Design and synthesis of novel sulphur containing anthracene-9,10-diones.

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Design and Synthesis of Novel Sulphur containing Anthracene-9,10-diones

By

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DECLARATION

The work described in this thesis was carried out in the organic and computational chemistry laboratories at the University of Wales, Swansea from September 1999 to September 2003, under the supervision of Professor John O. Morley.

This work has not previously been accepted in substance for any degree and is not being currently submitted in candidature for any degree.

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Date.....17/12/04.....

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ABSTRACT

It is well established that certain anthracene-9,10-diones such as Mitoxantrone **(24b)** and Amentantrone **(24a)** are potent anticancer drugs but suffer from the disadvantage that they are also cardiotoxic. Few sulfur containing anthracene-9,10-diones have been researched for their biological activity, and the current studies were aimed to synthesise a series of anthracene-9,10-dione derivatives containing both as substituted and as part of the ring system in an endeavour to conserve their anticancer activity but reduce their cardiotoxicity. A series of twenty four anthracene-9,10-diones were synthesized, seventeen of which have not been previously reported. Twelve of those derivatives contained sulfur and include the 1,4-bis(amino)-5,8-bis(sulfanyl)anthracene-9,10-diones, which were synthesised from the amination of 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione with selected amines followed by the subsequent reaction with thiolates. A ring derivative was also synthesised, 7-(aminoalkyl)-14H-naptho[2,3-a]phenothiazine-8,13-dione from the reaction of 1-(alkylamino)-4-hydroxyanthracene-9,10-dione and 2-aminothiophenol with boric acid. Semi-empirical molecular orbital methods were employed to model the anthracene-9,10-dione derivatives. To establish the most appropriate method for calculation a set of well known anthracene-9,10-diones were selected from the Cambridge crystallographic database and compared to computational data obtained using the AM1, PM3 and MNDO methods. All three methods give reasonable structures by comparison with the experimental data, when suitable constraints were applied to the optimisations. On balance the AM1 method was selected to model the synthesized anthracene-9,10-dione derivatives in this work. AM1 produced more accurate results for the nitrogen-carbon and oxygen carbon bond lengths. It also required no constraints when the simple sulfur derivatives were optimised. A series of reference calculations at the ab initio 6-31G** level were also carried out, to check veracity of the of the results obtained form the AM1 method.

The computational data highlighted two factors that may be significant in the biological activity of anthracene-9,10-diones. It appears that molecules that are biologically active contain alkylamino groups at the 1-and 4- position and have ionization potentials in the range of 7.7-7.9 eV. The computational data of the derivatives synthesized in this work shows many of them meet this criteria and therefore may possess anti-cancer activity, though this aspect of the research was not addressed in the current studies.

Acknowledgements

Firstly I would like to thank Professor John Morley for his support, guidance and patience, I know how trying I must have been! Thanks to the EPSRC for funding the research and keeping me off the streets.

Thanks to the people I have worked with, which include Owen "Welsh playboy" Guy, Stu "Injured" Whittaker, James "Ethanol" Padfield, Tom Matthews, Richard Morley, and Louise Warren. I would also like to thank the technicians and everyone else in the department who has helped along the way, which include Mas "IBM" Yousef, "Mad" Mike Oliver, Matt "Walkie talkie" Kennedy, Gareth Bullshit, Jo, Steve and "Big gay" Dave. Thanks to all the people that made Thursday my favourite day of the week! Craig, Gareth, Owen, Matt and all the other rugby boys.

Special thanks to the people that frequented 21 Maple Crescent and all the people I have met in Swansea along the way especially, Rohit "You got lovely eyes" Choudary, Sheepy " Have another tequila", Ginger Bromley, S4C Steve, Naked Rod and Karen Crayon.

Last but not least my family, especially mammy, daddy, sister, and yes, even my brother. Not forget getting the little man Louis and Claire.

One last thing, if anyone is thinking about working with anthraquinone, good luck you gonna need, this is one molecule I have grown to hate!

ABBREVIATIONS

AM1	– Austin Model 1
PM3	– Parametric Method 3
MNDO	-Modified Neglect of Differential overlap
DMF	– Dimethylformamide.
THF	– Tetrahydrofuran
HepG2 Cells	-Hepatic carcinoma cells
EDTA	-Ethylenediaminetetraacetic acid
DMSO	Dimethylsulphoxide
GLY	Glycine
HIS	Histidine
LYS	Lysine

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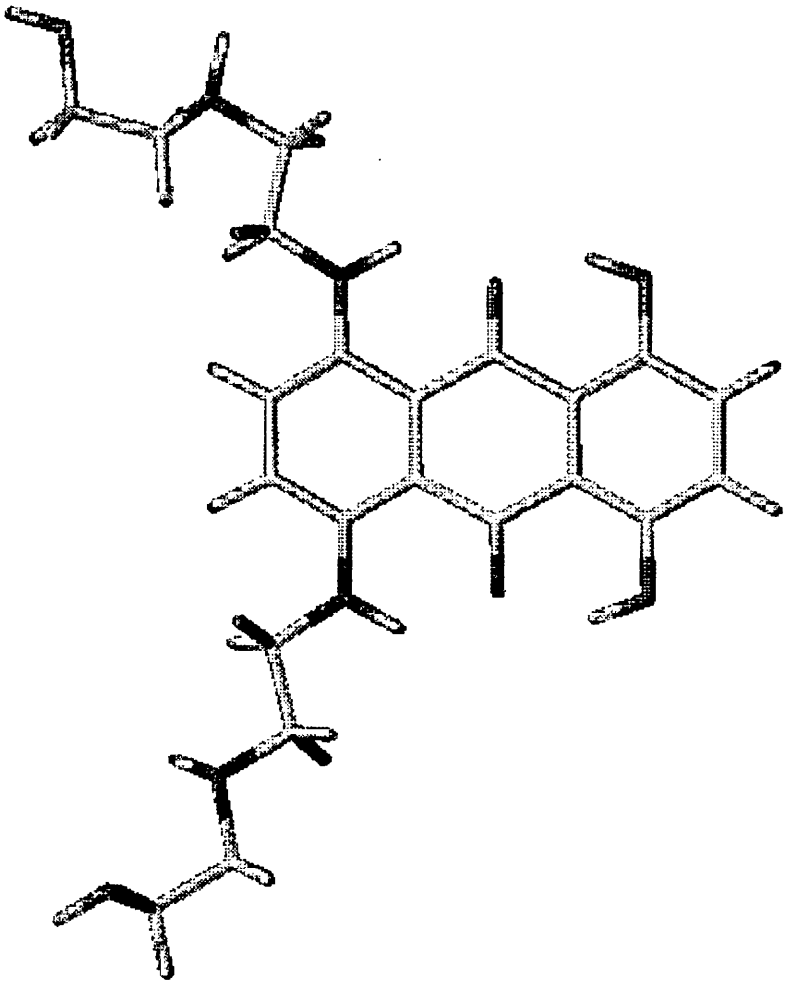
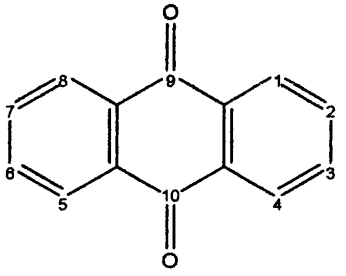
CHAPTER ONE



INTRODUCTION

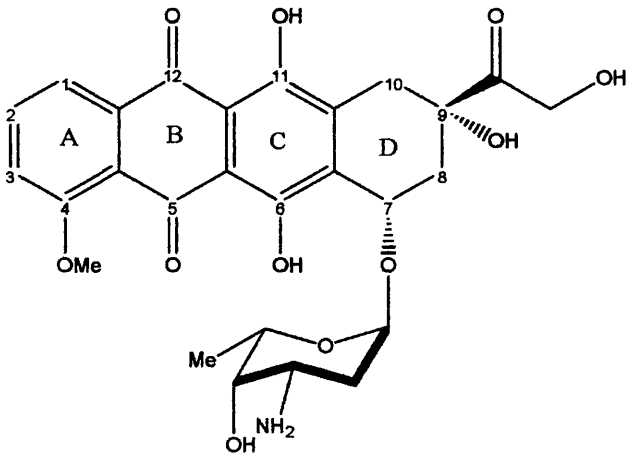
1.0General Background

Anthracene-9,10-dione (**1**) is the most important derivative of anthracene. It can be prepared by the condensation of phthalic anhydride and benzene or the direct oxidation of anthracene. It is a colourless crystalline solid, which melts at 285°C and has a molecular formula $C_{14}H_8O_2$; it also forms an important class of dyes.



(1)

Renewed interest in the chemistry of anthracene-9,10-diones follows the discovery of the anthracyclines. These compounds are an important class of clinical antitumor agents[1], with doxorubicin (**2**) being one of the most effective and widely prescribed anti-cancer agents in the field of chemotherapy. It is this class of anthracene-9,10-dione, which forms the basis of this thesis, and accordingly the use of anthracene-9,10-dione anti-cancer agents is reviewed in depth, followed by a brief account of the other applications of anthracene-9,10-diones including their use as dyes and pigments.



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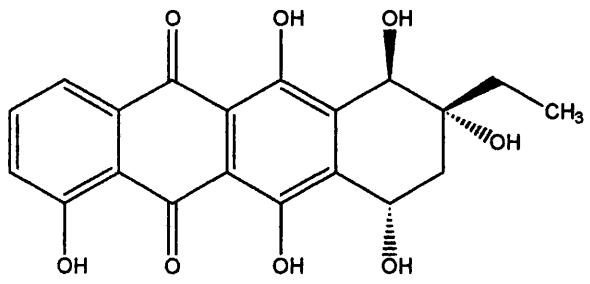
1.1 Anthracyclines

The anthracyclines were first discovered in the 1950's with the isolation of a number of pigmented substances from the strains of *Streptomyces pururascens* species[2] **(3-6)**.



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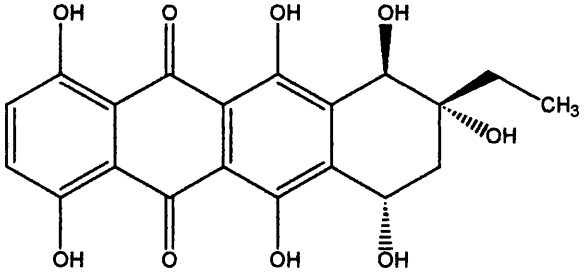

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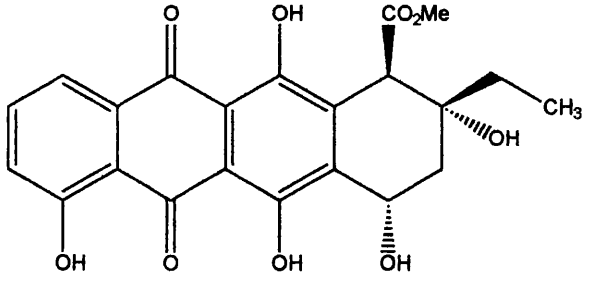

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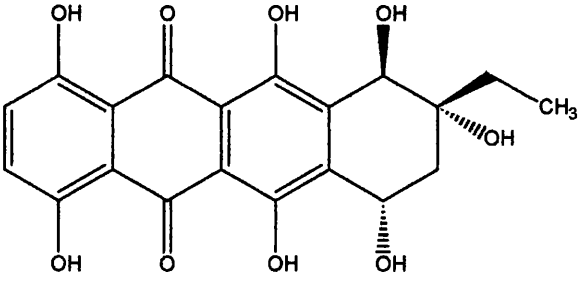

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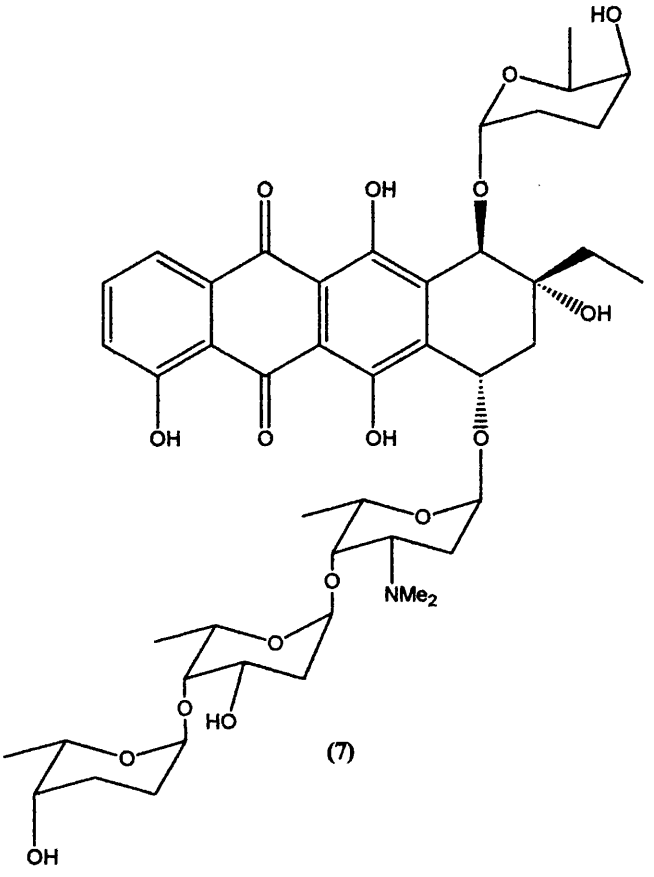
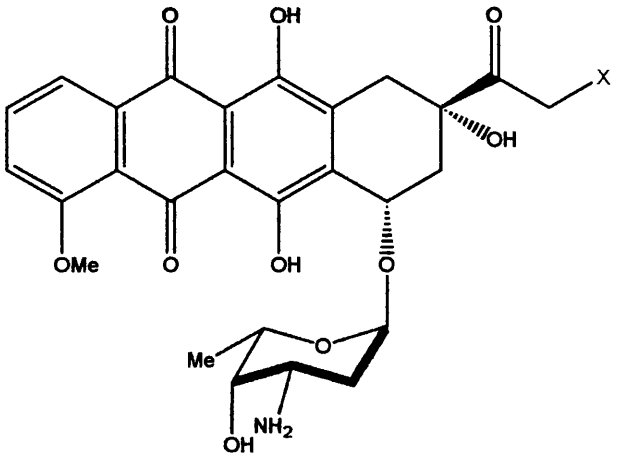
These compounds were characterised as glycosides of various substituted tetrahydronaphthacenequinones. Rhodomycin (7) was found to have antitumor properties but the compound was not suitable for human therapy due to the high toxicity in mice[3].



(7)

This discovery sparked further investigation into the biological properties of other derivatives in this class of compounds. In 1963, two research groups Faramitalia[4] and Rhone-Poulenic[4] independently described the isolation and characterisation of daunomycin (rubidomycin) **(8a)**, which, had antitumor properties and potential for treatment in humans. In 1969 the Faramitalia group isolated doxorubicin (Adriamycin) **(8b)** from *Streptomyces peucetius var. caesius*[4]. Extensive testing showed its superiority over daunomycin and this was confirmed in clinical tests against solid tumours. It is

interesting to note that a relatively small structural change, namely the introduction of a hydroxyl group, results in a large improvement of biological activity **(8 a-b)**.



(8)
(a) X=H Daunomycin
(b) X=OH Adriamycin

There has been in excess of 2000 anthracyclines synthesised to date however none have superseded the anti tumour activity of Daunomycin and Adriamycin.

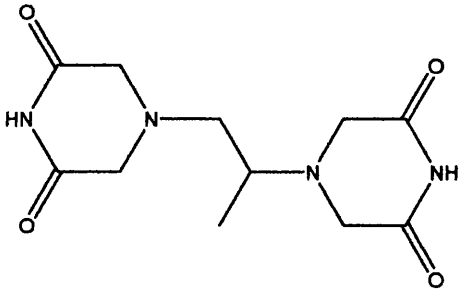
1.1.1 Daunomycin

The first report on the anti tumour activity of daunomycin indicated exceptional pharmacological properties. The drug was tested mainly on leukaemias, Hodgkin's disease, lymphosarcoma and reticular cell sarcoma. However toxic side effects were noted in patients: they were stomatitis, alopecia, bone marrow suppression and gastrointestinal disturbances. The most serious limiting factor was recognized as the dose dependent cardiotoxicity.

1.1.2 Adriamycin

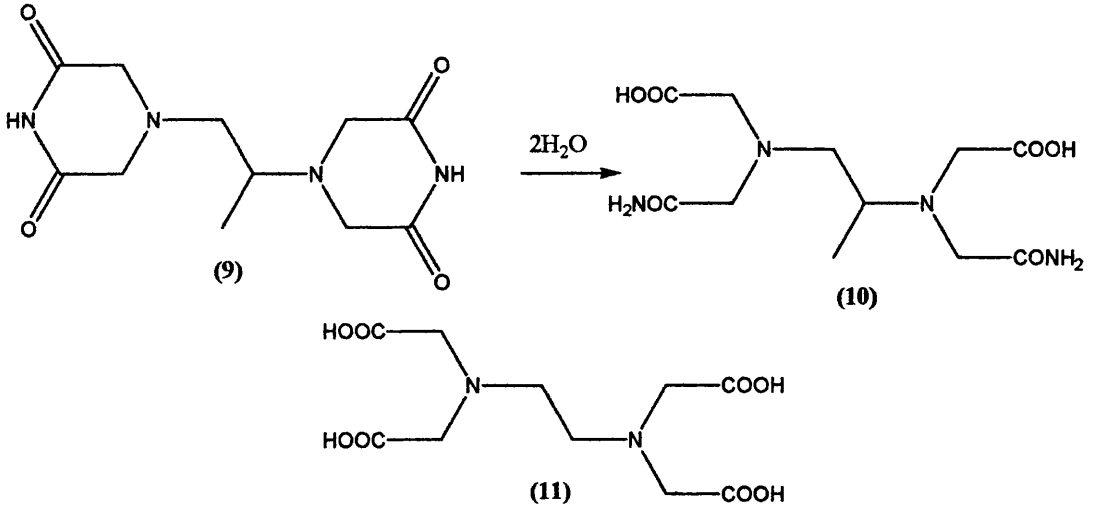
Adriamycin exhibited the same kind of inhibitory effect as daunomycin, but was generally more potent. Clinical trials confirmed the early promise of this valuable agent, which proved to be effective as a single agent or when combined with other drugs. The results of extensive clinical trials proved that the drug had a wide-ranging spectrum of activity, though the only real limiting factor was again the cardiotoxicity.

Cardiotoxicity is attributed to the formation of a drug iron complex, which can lead to harmful free radicals, due to the complex's ability to undergo reversible oxidation. These free radicals damage the cardiac muscles, which are deficient in protective antioxidants. In an attempt to counteract this cardiotoxicity the anthracyclines have been administered with drugs that combat this harmful side effect. Such cardioprotective agents include dexrazoxane[5](ICRF-187, Zinecard, **9**), which works by competing for iron



(9)

This is affected when the molecule is hydrolysed to its ring opened form **(10)** *in vivo*, which has a high affinity for metal ions, in a similar manner to EDTA **(11)**.



(9) (10) (11)

1.1.3 Classification on basis of structure or mode of action

Anthracyclines are classified upon the similarity in structure and the unique groups, which they may possess. Anthracyclines can also be classified into those, which bind intercalatively to DNA, and those, which do not. There are two classes of intercalative anthracyclines, class I which inhibit both DNA and RNA equally and class II which inhibits RNA synthesis 200 to 1300 times more effectively than DNA synthesis. Class I\& II, can be further divided on the ability of the molecules to bind to DNA. The anthracyclines can also be further classified on their ability to form a non-cleavable complex with topoisomerase II and DNA.

1.1.4 Mode of action

Despite their extensive clinical utilisation, the mode of action is subject to controversy[6]. It has been discovered that anthracyclines rapidly enter cells, gather in the nuclei and bind to DNA. Anthracyclines can bind to DNA in two ways, into the minor groove or intercalatively. Minor groove binding is a result of DNA being twisted into its double helical form. Because the DNA bases in the double helix are not twisted 180° to each

other, two grooves appear, the minor and the major grove (Figure 1-1). The minor groove is where anthracyclines preferentially bind to DNA.

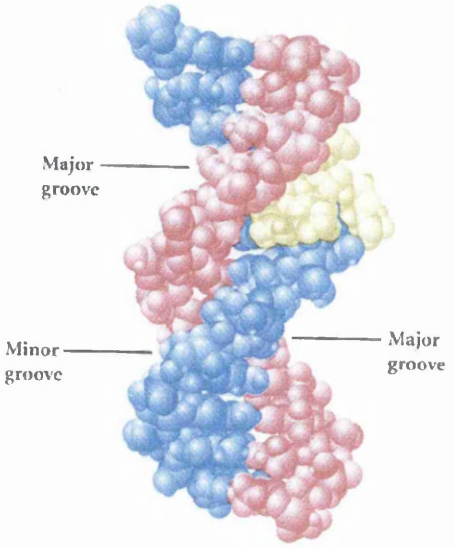


Figure 1-1. Minor groove binding of anthracyclines (yellow) with DNA (red & blue).

Anthracyclines can also bind to DNA intercalatively. As the chromophore is a planar polycyclic molecule it has the ability to slip in between the DNA base pairs[7], this is known as intercalation (**Figure 1-2**).

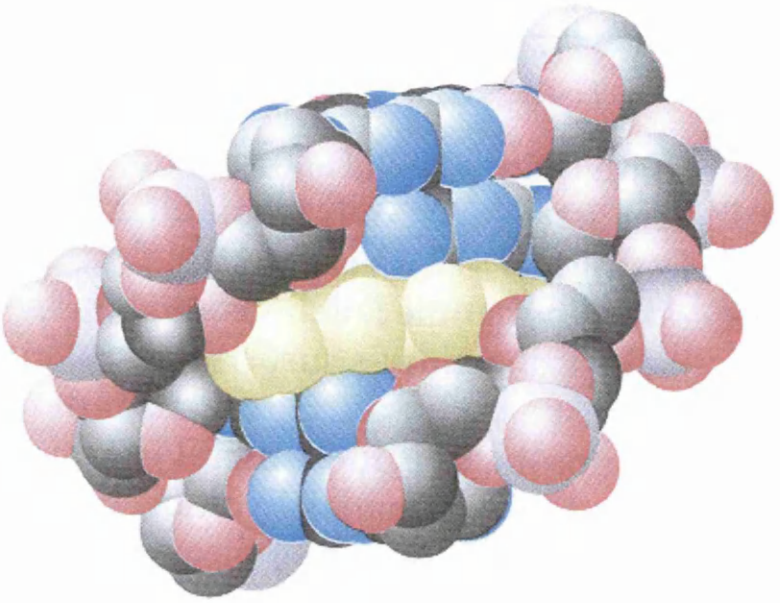


Figure 1-2. Intercalation of anthracyclines (yellow) into DNA (red & blue)

Minor grove binding and intercalation can stop the DNA from unwinding (in the same way something getting stuck in a zipper would cause it to jam or a stick in a bicycle wheel). If the DNA cannot unwind the cell cannot replicate and will therefore die. Minor grove binding and intercalation cannot account solely for the potency of these drugs, though the process is thought to act as some sort of "anchoring mechanism"[8] until a cell killing event occurs.

Once bound to the DNA they can further interfere with cell transcription and replication in two ways.

I. Anthracyclines interfere with the normal mode of action of topoisomerase II, which is an important class of enzyme that is essential for cell replication. It is involved in the unwinding of a cells DNA from its double helical form into single strands.

   Anthracyclines form an un-cleavable complex with the enzyme. This complex is not

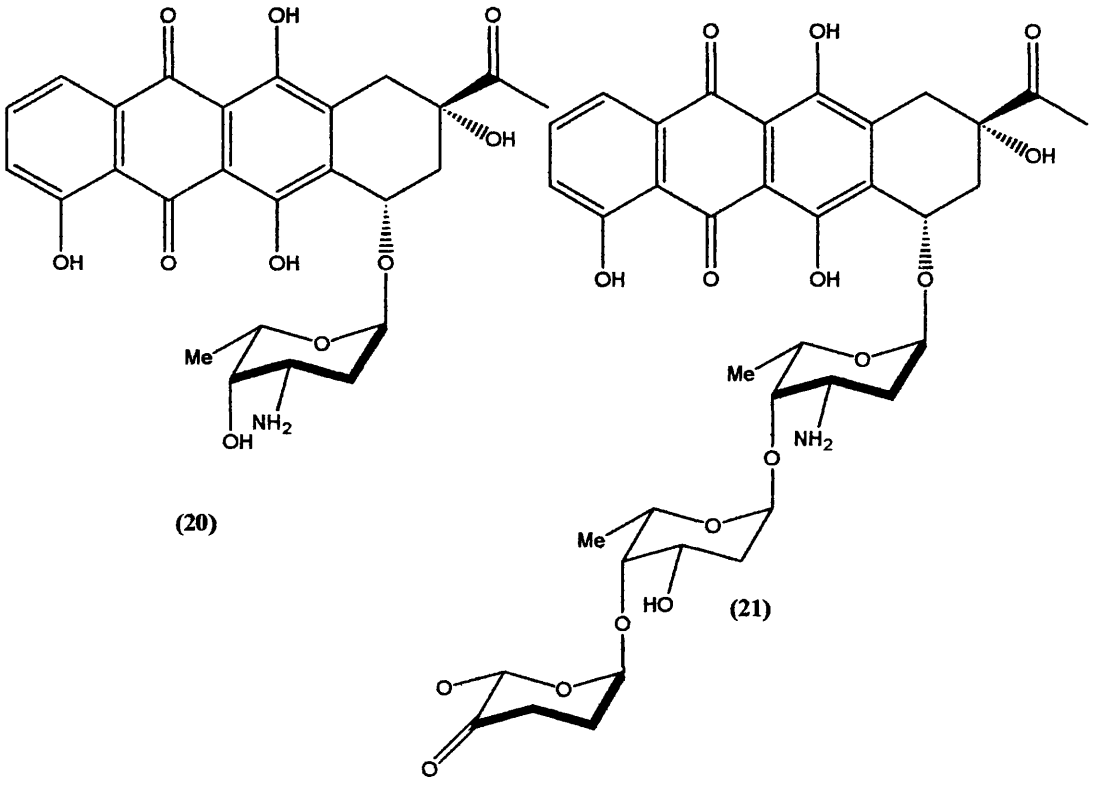
sufficient to insure cell death, however once the complex is formed the enzymes mode of action is modified. Instead of just unwinding and separating the DNA strands it now also causes breaks in the DNA strand. These strand breaks are then sensed by the cell via a series of stress associated pathways, the cell arrests and apoptosis (see p26 section 1.2.9) occurs[9].

II. Anthracyclines can also form damaging free radicals via enzymatic reduction. A one-electron reduction produces a semiquinone and two-electron reduction gives a hydroquinone. The semiquinone radical is capable of transferring its electron to molecular oxygen to form a superoxide radical, which is converted to hydrogen peroxide via a disproportionation process. Damage occurs when the hydrogen peroxide comes into contact with reduced metal ion such as copper or iron, which then leads to formation of the hydroxyl radical. This highly reactive hydroxyl radical is responsible for oxidation of fatty acids, degradation of proteins and DNA damage via Fenton type reactions. This process is why these compounds are so cardiotoxic, as the heart is susceptible to free radical damage because of a deficiency in protective enzymes. Free radicals can also form covalent bonds with DNA, which in effect stabilize the double helix preventing DNA from unwinding. For a cell to replicate the DNA it must be unwound into its single form. If a cell cannot do this it cannot perform the basic functions that keep it alive and it therefore dies.

1.1.5 Recent developments in the field of anthracyclines

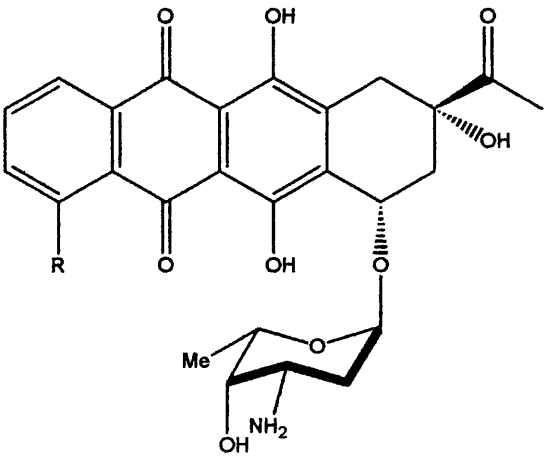
Despite the widespread use of Adriamycin and Daunomycin for thirty years, there have been few improvements to the drugs, despite extensive research aimed at developing anthracyclines with greater activity and lower cardiotoxicity. This research has lead to

the synthesis of over 2000 analogues. Some of the derivatives including Cariomycin (or Carubicin **(20)** [10] originally developed in the Soviet Union in the 1980's, Aclacinomycin A (or Aclarubicin **(21)**[11] that were both marketed in France and Japan for the treatment of leukaemia.



(20) **(21)**

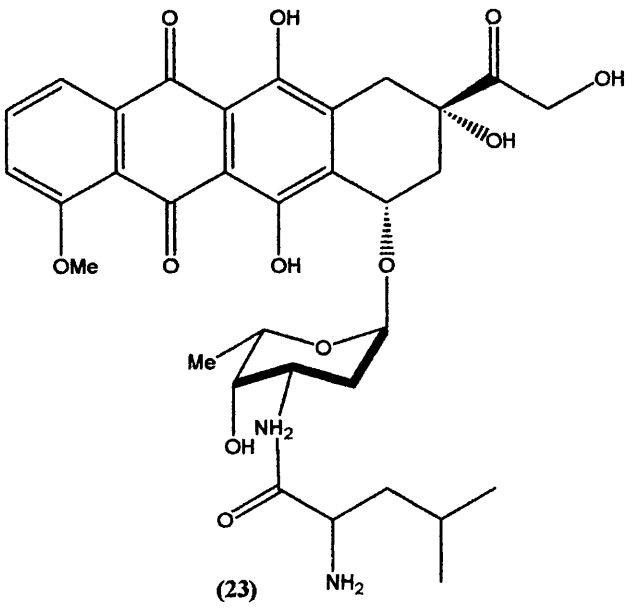
Other marketed compounds include daunorubicin benzoylhydrazone (Zorubicin or Rubidazone®)[12] and Idarubicin hydrochloride (Idamycin) **(22b)**, which differs from the parent drug daunomycin **(22a)**, by the absence of a methoxy group at position 4.



(22)
(a) R=OMe Daunomycin
(b) R=H Idrarubicin

The absence of this group increases the drugs lipophilicity and its DNA binding ability. One approach to improve the effectiveness of doxorubicin has been to improve its delivery to tumours, using drug-carrier technology. This approach can be classified in two ways, depending on whether there is a specific recognition of a tumour by the carrier or simply preferential distribution. The advantages of this technology are less of the drug is required, resulting in less damage to non-tumour cells and fewer side effects.

For example, encapsulation of Adriamycin or daunomycin in liposomes has been used and there have been three commercial products marketed as Lipodox ®, Doxil ®and DaunoXome ®. More interesting, however, is the development of conjugates of doxorubicin with amino acids such as N-L-leucyldoxorubicin **(23)**, which has superior efficiency over doxorubicin.

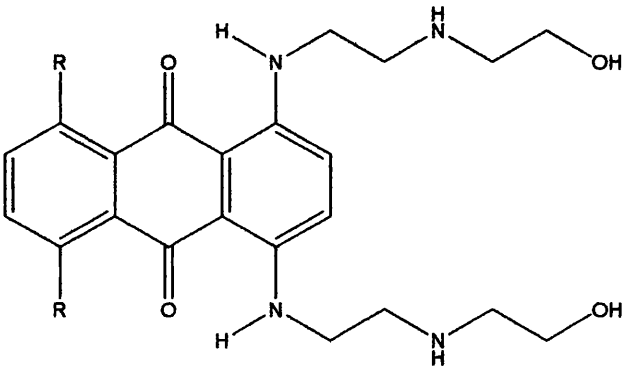


(23)

These drug conjugates work on the principle that many cancers exhibit elevated levels of peptide hormone receptors to which the amino acid has a high affinity for. More recent developments include the discovery that tumour cells require an intensive network of blood vessels to supply their ever-increasing size. This blood vessel network has identification markers attached to the blood vessel cell surfaces and are known as antigens. These antigens are proteins and are different for each type of cell they are attached to they act in the same way as a postcode. Tumour cells were found to express an antigen known as the, α_1 integrin, and peptides have been found that selectively bind to this antigen, such as Arg-Gly-Asp (RGD) or Asn-Gly-Arg. These peptides when attached to Adriamycin were found to greatly enhance the drug's efficiency[13].

1.2 Simpler Anthracene-9,10-dione derivatives

Following extensive research into the anthracyclines it was proposed that the amino sugar component was responsible for the cardiotoxicity[14]. It was therefore suggested that a simpler planar molecule with properly selected amino or amino alkyl side chain could eliminate the cardiotoxicity[15,16]. It was found that the 1,4-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-anthracene-9,10-dione **(24a)** (Amentantrone) had good activity against leukaemia, confirming this suggestion. Further investigation led to the discovery that substitution at the 5 and 8 positions of the anthracene-9,10-dione chromophore with hydroxyl group improved the antineoplastic activity. Thus 1,4-dihydroxy-5,8-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-anthracene-9,10-dione **(24b)** (Mitoxantrone) requires just one tenth of the dose[17] of that required by 1,4-bis[[2-[(2-hydroxyethyl)amino]ethyl]amino]-anthracene-9,10-dione **(24a)** derivative for antineoplastic activity.



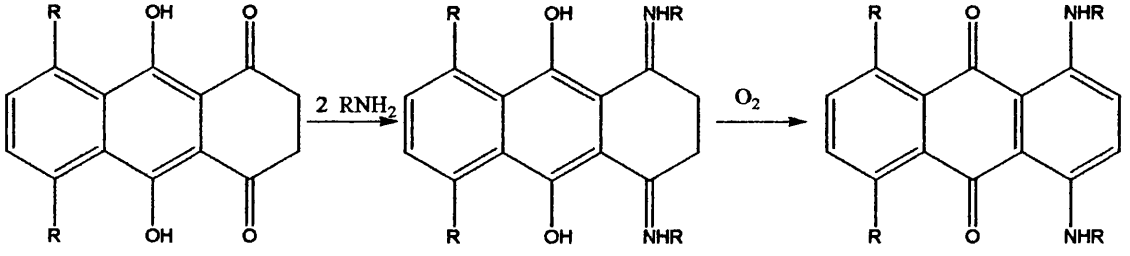
(24)
(a) R= H Ametantrone
(b) R= OH Mitoxantrone

It was later discovered that the increased activity of Mitoxantrone was limited due to the ten fold increase in the cardiotoxicity and therefore the effectiveness of Mitoxantrone and

Amentantrone is similar at the optimal dose[18]. Although these compounds are cardiotoxic, they are less cardiotoxic than anthracyclines. Clinically they are used instead of the anthracyclines if the patient has a history of cardiac problems. The most common dose-limiting factor for Mitoxantrone is the myelosuppression, which is a decrease in the ability of the blood cell-producing tissues of bone marrow to produce all types of blood cells[19] and in most cases it is not severe or life threatening. Acute toxicities include nausea, vomiting, stomatitis and alopecia but these are less severe when compared with doxorubicin. Other effects include flu like symptoms and the blue discoloration of the skin, fingernails and the urine[20].

1.2.1 Synthesis

Preparation of the 1,4-bis[(amino alkyl)amino] anthracene-9,10-diones is achieved by the condensation of the appropriate leuco or reduced (leuco is the term used in the dye industry) form of the anthracene-9,10-dione **(25)**, with an excess amount of amine at 50-55°C followed by subsequent air oxidation[21]



Scheme 1-1. Synthesis of 1,4-bis(aminoalkyl)anthracene-9,10-diones

The oxidation of **(26)** takes place with relative ease and it is difficult to isolate, even if the reaction has been carried out under a nitrogen atmosphere.

1.2.2 Structure vs. Biological activity

After detailed structure activity results on the 1,4-bis[(amino alkyl)amino] anthracene-9,10-diones the following observations were made.

I. Anthracene-9,10-dione its self has no activity.

II. Removal of the terminal hydroxyl group on the side chain of **(24a)** and **(24b)** leads to retained activity but at a lower level.

III. The distance between the two nitrogen's on the side chain of **(24a)** and **(24b)** is an important factor, the optimum being two carbons apart.

IV. Insertion of additional ethyl amino units into the side chain drastically reduces activity, indicating that additional basic centres and lengthening of the side chain is undesirable.

V. The nitrogen in the centre of the side chain plays an important role in activity. No activity is noticed when this nitrogen is replaced by a methylene unit or another atom such as sulphur.

VI. Hydroxyl substitution at the 5 and 8 positions of the anthracenedione greatly improves activity.

1.2.3 Mode of action

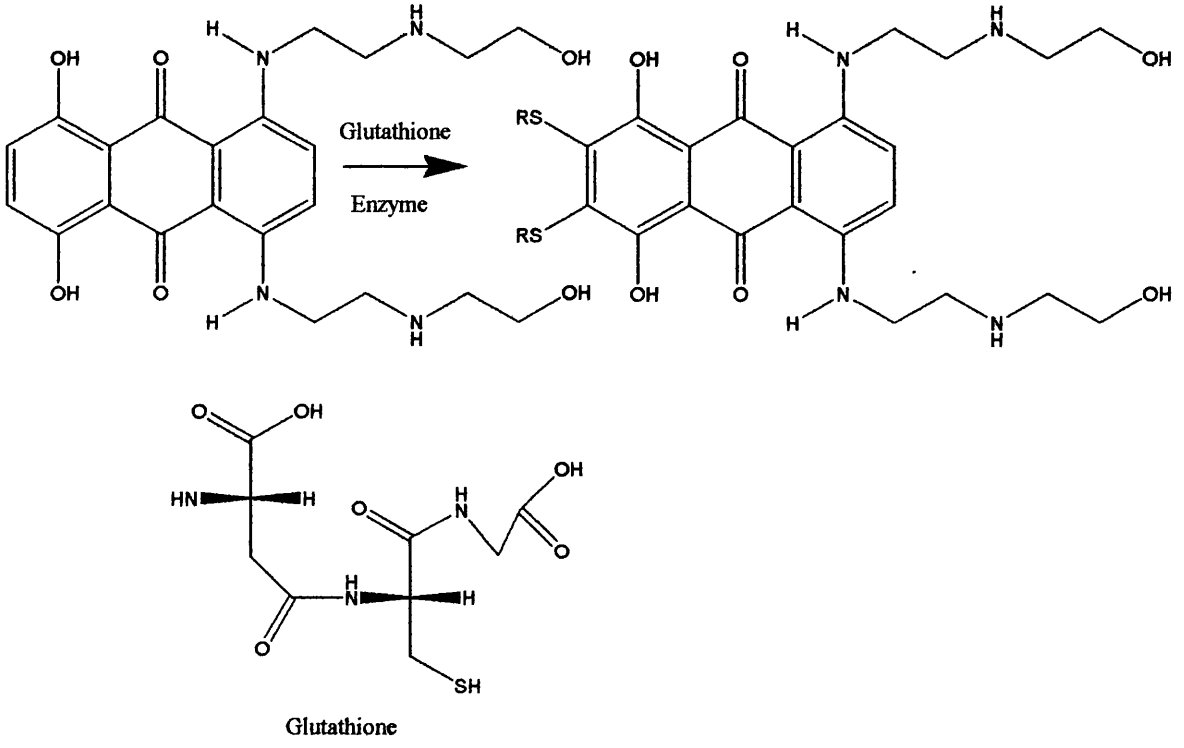
The mode of action for these compounds is very similar to that of the anthracyclines (see section 1.1.4, p7) and as with the anthracyclines the specific mechanism is unclear. It is probably a combination of the following factors (for more detail see section 1.1.4, p7)

I. They are flat and planar molecules and therefore can intercalate into DNA.

II. They can under go a reversible oxidation process resulting in formation of free radicals[22, 23].

III. They can interfere with the normal mode of action of topoisomerase II[24, 25].

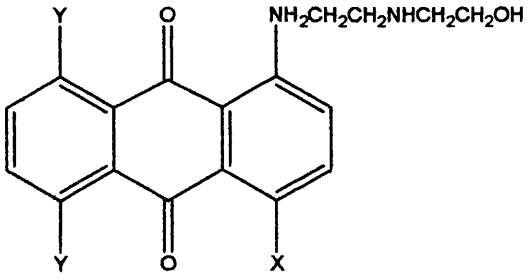
IV. The drug Mitoxantrone can undergo reaction with glutathione via an enzyme-mediated reaction (Scheme 1-4). Glutathione is a tripeptide that is involved in protecting DNA and RNA by mopping up reactive species such as free radicals. Therefore the loss of glutathione can lead to cell death[26].



Scheme 1-2. Enzyme catalysed reaction of glutathione with Mitoxantrone

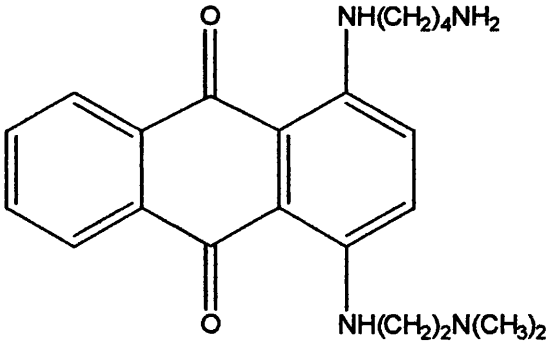
1.2.4 Unsymmetrically substituted 1,4-bis[(amino alkyl)amino] anthracene-9,10-diones

Several un-symmetrically substituted analogues that hold the "Mitoxantrone side-arm" at position 1 and a hydroxyl or amino substituents at position 4 also show high anti tumour activity **(27a-b)**



(27)
(a) Y= OH or H
(b) X=OH or NH_2

The introduction of different amino alkyl groups at position 1 and 4 has lead to compounds with good *in vitro* activity, in particular analogues such as **(28)**, although this compound was inactive *in vivo*[18].

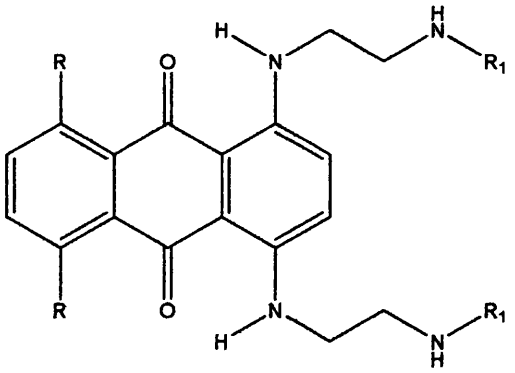


(28)

1.2.5 Metal chelating anthracene-9,10-diones[27]

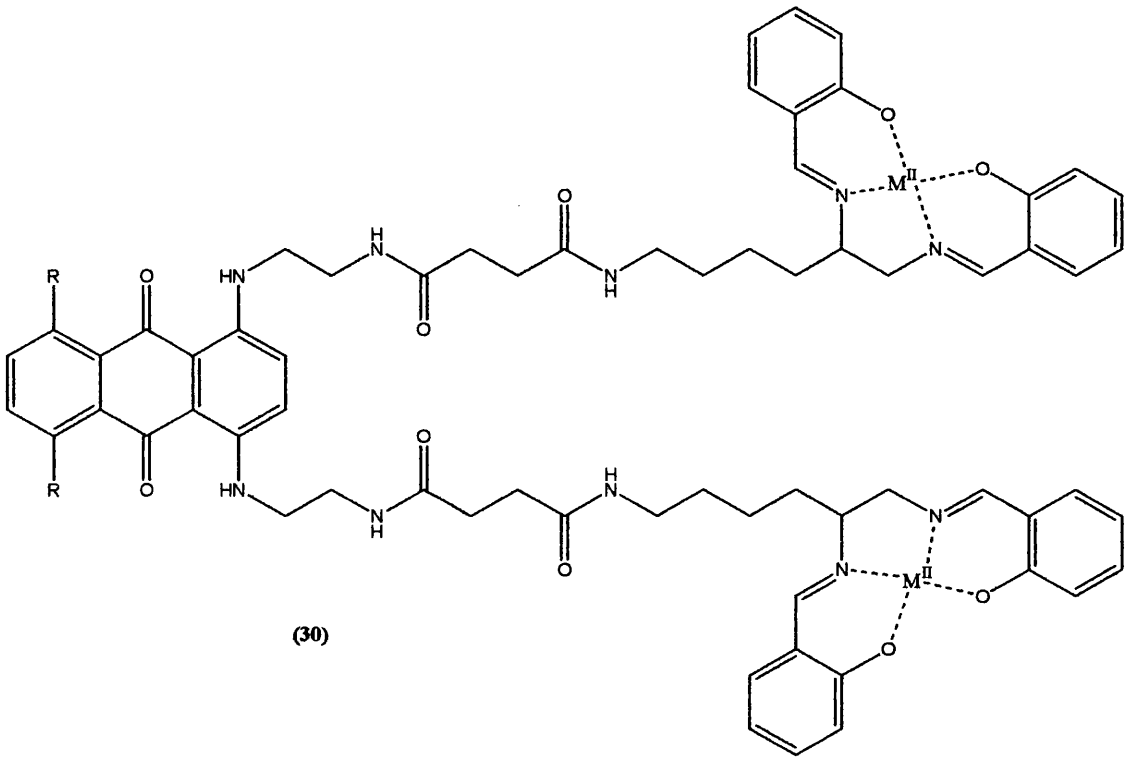
With the uncertainty about the mode of action, attempts were made to synthesise anthracene-9,10-diones that would have improved efficiency to form free radicals to see if their formation was an important factor in the anticancer activity. Anthracene-9,10-diones were therefore synthesized that had side chains, which had, metal chelating

properties such as the peptide Gly-L-His-L-Lys or Gly-Gly-L-His **(29 a-b)** which have a high affinity for copper and iron[28].



(29)
(a) R=OH R_1=Gly-L-His-L-Lys
(b) R= OH R_1=Gly-Gly-L-His

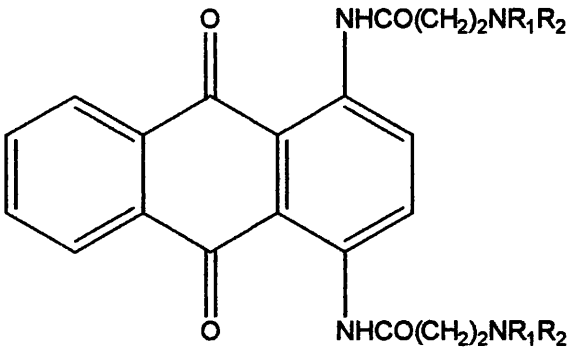
Anthracene-9,10-diones of **(29a)** showed excellent *in vitro* activity but was inactive *in vivo*, however **(29b)**, had good *in vitro* activity and good *in vivo* activity against leukaemia cells in mice. The activity of **(29b)** is slightly less active than Mitoxantrone **(24b)** the parent drug but it is less toxic. The poor activity *in vivo* of **(29a)** has been attributed to the poor cell penetration and degradation of the molecule. Anthracene-9,10-diones were also synthesised with chelating ligands such as **(30)**[29] but these had no *in vitro* activity, because the large side chain has altered the DNA binding properties.



(30)

1.2.6 Amidoanthracene-9,10-diones

In an attempt to assess the role of the primary nitrogen in the side chain of Mitoxantrone and Amentantrone, the amidoanthracene-9,10-diones **(31a,b)** were synthesised. Modification of the nitrogen atom attached directly to the anthracene-9,10-dione nucleus would be expected to exert significant influence on the electron density in the anthracene-9,10-dione nucleus, and thus the π-electron interaction with DNA bases and also the electron-accepting ability of the quinone system. Therefore nitrogen atoms bonded to the anthracene-9,10-dione should have important consequences on the anti-tumour activity and cardiotoxicity of the compounds.



(31)
(a) R_1=H R_2= CH_2CH_2OH
(b) R_1=R_2=CH_3

Due to acylation, the nitrogen's attached to the anthracene-9,10-dione ring will have lost their basic character causing a strong reduction of π-electron density in the quinone moiety[30]. The *in vitro* testing of the amidoanthracene-9,10-diones showed considerable activity, comparable to that of Amentantrone. Thus modification of the primary nitrogen in the side chain does not affect activity. As with Mitoxantrone, the optimal number of carbons between the nitrogen's in the side chain is two, but unlike Mitoxantrone the ethanolic side chain in **(31a)** has lower activity compared to the methyl derivatives **(31b)**. *In vivo* testing, however, revealed poor activity, and in higher doses both compounds were toxic.

Further research showed that the amidoanthracene-9,10-diones mode of action was different *in vitro*. The acylation of the ring nitrogen atoms causes conformational constraints and therefore the side chains are less flexible leading to differences in the interaction of the compounds with DNA especially with the base nitrogen and electrostatic oxygen atoms that are in the plane of the bases[31].

Research has show that the amidoanthracene-9,10-diones act on g-quadruplex of DNA[32], which is a secondary structure adopted by DNA sequences that are rich in the base guanine. Four guanine bases can associate in a planar, hydrogen bonded assembly called a g-tetrad (or quartet) where each guanine simultaneously accepts and donates two hydrogen bonds[33]. Successive layers of g-tetrads allow single stranded DNA to adopt a folded back structure called the g-tetraplex (or g-quadruplex) (Figure 1-3).

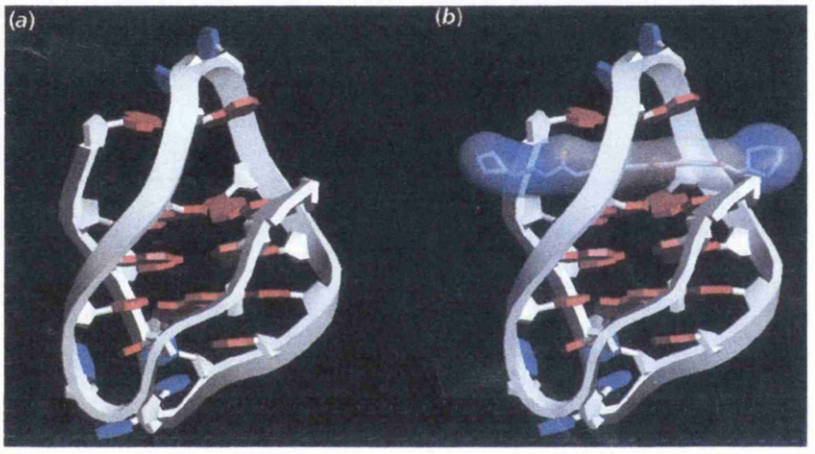


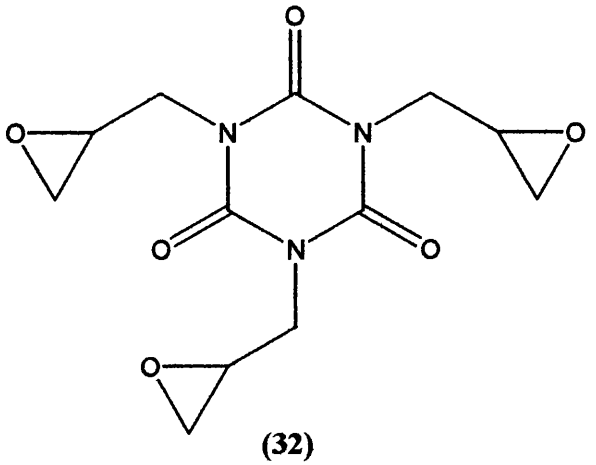
Figure 1-3. (a)A single DNA strand folded into a g-quadruplex (b) the DNA g-quadruplex stabilized by an amidoanthracene-9,10-dione.

The g-quadruplex is a structure found in the telomere regions of DNA, telomeres are the ends of chromosomal DNA. Their function is to protect the important information stored in a DNA strand, just like pieces of plastic on the end of a shoelace protects it from fraying. Telomeres are around 50-200 bases long and are rich in guanine. Each time a cell divides the telomere becomes shortened. Once the telomeres reach a critical length

the cell enters a non-divide state called senescence, which leads to irreversible cell death. This shortening of the telomeres is one of the reason why a cell ages. However, shortening of telomeres doesn't occur in cancerous cells, because of the presence of the enzyme telomerase, which repairs the telomeres and therefore prevents them from shortening, thus making the cell immortal. The enzyme telomerase has been found in 85-95% of all tumours and is used as a diagnostic marker for cancer; telomerase is therefore an obvious target for cancer drugs. The fact that amidoanthracene-9,10-diones bind to the g-quadruplex is not sufficient to account for its activity, but telomerase requires DNA to be in its single stranded form with no secondary structure, the g-quadruplex is an example of such a structure, amido anthracene-9,10-diones initiate the formation of the g-quadruplex's and stabilize the structures once they have formed, therefore telomerase is inhibited by amidoanthracene-9,10-diones. If telomerase cannot maintain the length of the telomeres cancerous cells are no longer immortal and therefore die. Currently the amidoanthracene-9,10-diones are too toxic to normal cells and are therefore not yet viable drugs for the treatment of cancer.

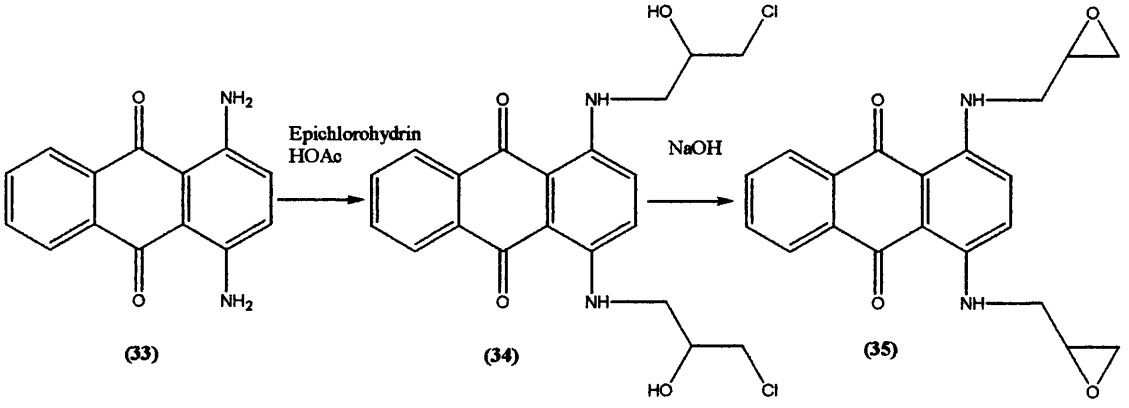
1.2.7 1,4-Bis-(2,3-Epoxypropylamino)-9,10-anthracene-9,10-diones

Teroxirone[34] **(32)** is a 1,3,5 triazine molecule, which contains alkylating epoxide moieties in its amino side chains.



(32)

Teroxirone is a diverse antineoplastic drugs that contain reactive groups capable of covalently modifying a variety of biological molecules and therefore have a different mode of action compared to the previously mentioned anthracene-9,10-dione drugs. Thus, a single molecule containing an anthracene-9,10-dione moiety with alkylating epoxide groups could prove beneficial.[35]. In order to achieve this 1,4-bis-(2,3-epoxypropylamino)anthracene-9,10-dione was synthesised **(35)**.



(33) (34) (35)

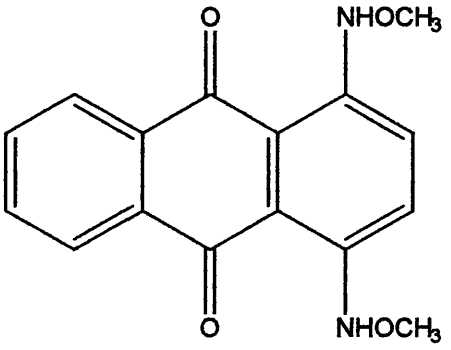
Scheme 1-3. Synthesis of 1,4-bis-(2,3-epoxypropylamino)anthracene-9,10-diones.

Compound **(35)** contains both a planar intercalating moiety and an alkylating functional group, therefore it should be able to intercalate into the DNA backbone and react with

important cellular molecules. Initial testing has shown that **(35)** show potential activity and selective cytotoxicity.

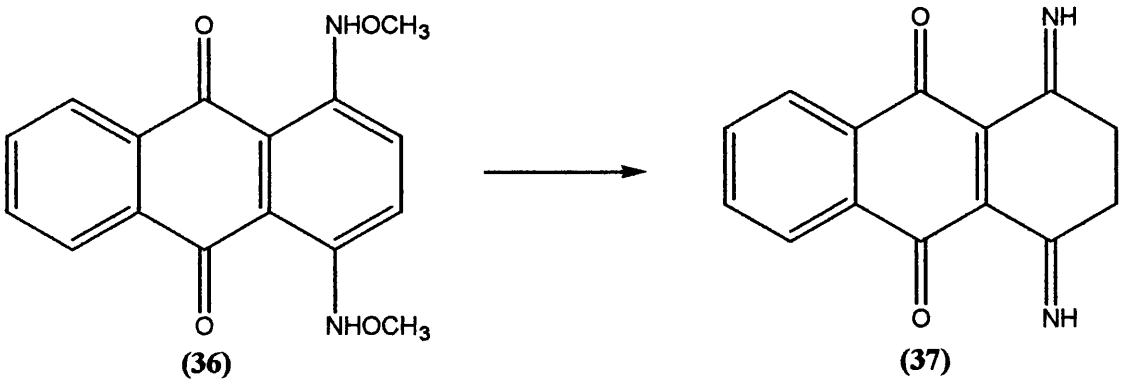
1.2.8 1,4-Bis-[methoxyamino]anthracene-9,10-diones

Methoxyamine analogues **(36)** have been prepared, which could act as bio reductive alkylators under cellular conditions[36].



(36)

It has been suggested that intermediate **(37)** might be formed by enzymatic sequential two electron reduction to **(36)** followed by reductive elimination of the N-O bonds, which might be expected to undergo attack by nucleophilic cellular components to form covalently bonded species. An event of this type could lead to cellular destruction of hypoxic tumours[36].



(36) (37)

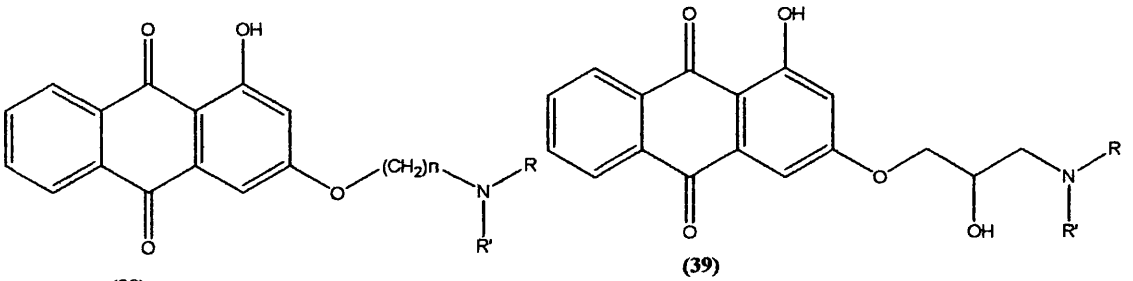
Figure 1-4. Enzymatic reduction followed by reductive elimination of the N-O bond

Synthesis is achieved by reaction of 1,4-difluroanthracene-9,10-dione with methoxylamine in DMSO[36].

1.2.9 1,3-Dihydroxy-anthracene-9,10-diones derivatives[37]

This class of anthracenediones **(38,39)** is thought to directly induce apoptosis of a cell. Apoptosis, or programmed cell death, is a normal component of the development and health of multicellular organisms. Cells die in response to a variety of stimuli, and during apoptosis this is done in a controlled and regulated fashion. This makes apoptosis distinct from other forms of cell death such as cell injury, which is due to mechanical damage or exposure to toxic chemicals. Cell injury has a characteristic series of changes, the cell and their organelles, swell because the ability of the plasma membrane to control the passage of ions and water is disrupted, the cell then bursts and the contents leak out, leading to inflammation of surrounding tissues[38]. Apoptosis, by contrast, is a process in which cells play an active role in their own death, which is why apoptosis is often referred to as cell suicide[39]. An example of apoptosis is the formation of the fingers and toes of the foetus, which requires the removal of the tissue between them or the loss of a

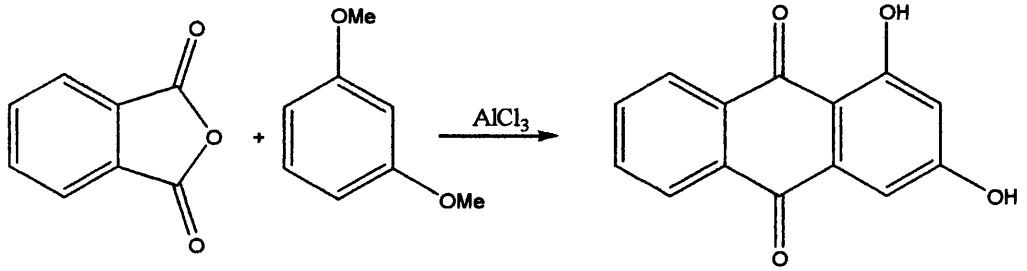
tadpole's tale, as it becomes a frog.



(38)
(a) n=2 R'=R=H
(b) n=2 R'=R=C_2H_5
(c) n=2 R'=R=CH_3
(e) n=3 R'=R=H
(f) n=3 R'=R=C_2H_5
(g) n=3 R'=R=CH_3

(39)
(a) R'=R=H
(b) R'=R=C_2H_5
(c) R'=R=CH_3

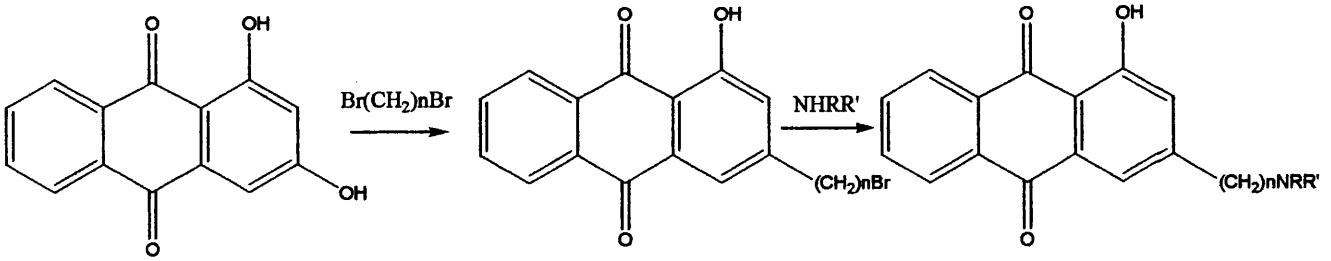
The simple 1,3-dihydroxy-anthracene-9,10-dione **(40)** is synthesised from the condensation of phthalic anhydride with 1,3-dimethoxybenzene.



(40)

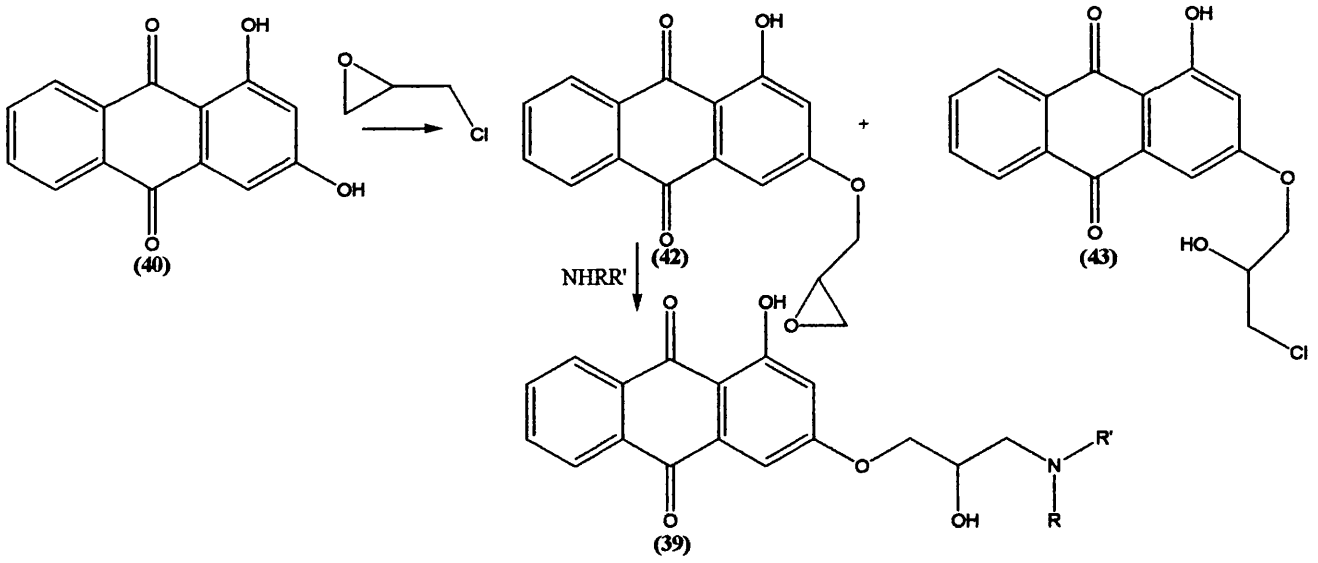
Scheme 1-4. Synthesis of 1,3-dihydroxy-anthracene-9,10-dione.

Compounds **(38)** in turn are synthesised from the reaction of ω-dibromoalkane with **(40)** in the appropriate solvent to give **(41)**, which is then aminated with the appropriate amine.



(40) (41) (38)

Compounds **(39)** in turn are synthesised from the reaction of **(40)** with sodium hydroxide in 2-propanol and epichlorohydrin to give **(42)** as the major product and **(43)** as the minor. The product **(42)** is then ring opened with the appropriate amine to give **(39)**.

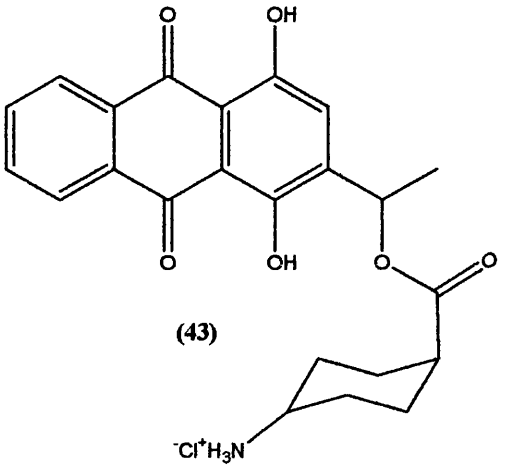


(40) (42) (43) (39)

Initial testing on cancer cells indicates that compound **(38g)** causes fragmentation of DNA in the cells, which is generally used to characterise cell death by apoptosis[40]. The mechanism of action is unclear and requires further research.

1.2.10 Anthracycline mimics

In attempt to combine the anthracyclines with the simpler structure of the Mitoxantrone compounds such as **(43)** [41] were synthesised. They consist of 1,4-dihydroxyanthracene-9,10-dione with a doxorubicin sugar mimic attached at position 3.

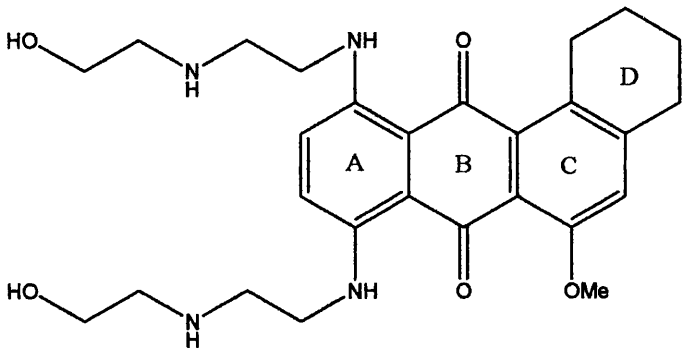


(43)

The preliminary evaluation *in vitro* showed activity against leukaemia cell lines, though the activity is approximately one order of magnitude less than Daunomycin, which is surprising for such a simple analogue.

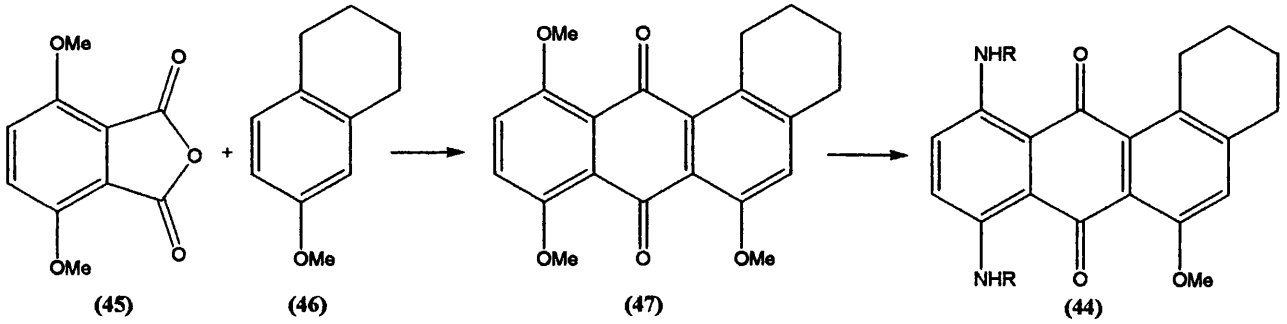
1.2.11 Tetrahydrobenz[a]anthracene-9,10-dione derivatives

This class of compound **(44)** mimics the type of functional groups and stereochemistry found in the anthracyclines.



(44)

Introduction of the D ring introduces a non-polar zone, which should influence intercalation into DNA[42]. Synthesis is achieved from the reaction of 3,6-dimethoxy phthalic anhydride **(45)** with 1,2,3,4,-tetrahydronapthalene **(46)**, followed by demethylation of **(47)**, subsequent reduction, and then amination.



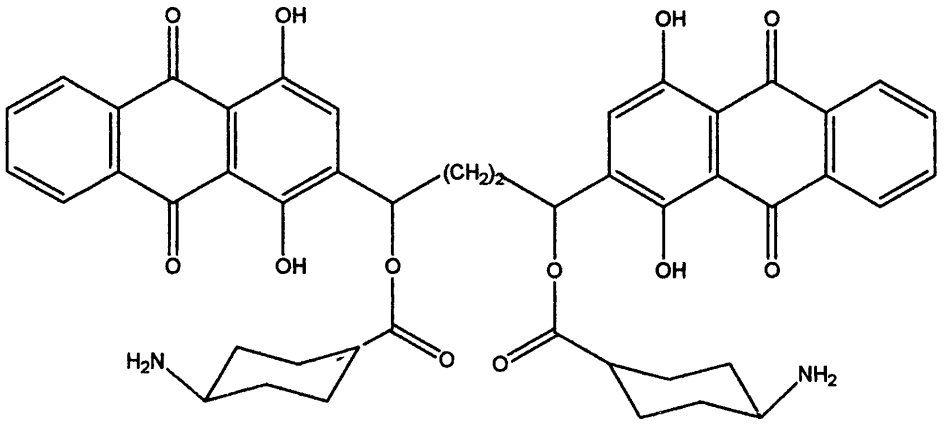
(45) (46) (47) (44)

Testing showed that compound **(44)** demonstrated significant antileukemic activity[42].

1.2.12 Bisanthracene-9,10-dione derivatives[43]

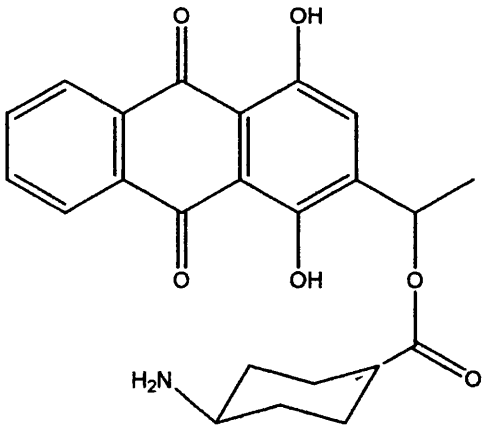
The concept of bis-intercalating agents evolved from the view that combining two intercalating agents into one molecule would produce a more efficient DNA intercalator with double the capacity. Bifunctional ligands such as **(48)**, in which both anthracene-

9,10-dione molecules could intercalate simultaneously, should have greater selectivity due to the increased size of the binding site and as a consequence be less toxic.

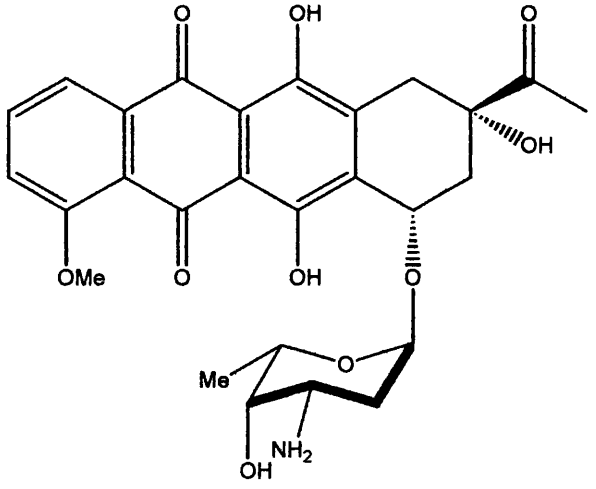


(48)

In the designing such bisintercalators it was proposed that the simpler anthracycline analogue **(49)** would be appropriate.



(49)



(50)

The mimic **(49)** had already shown activity similar to daunomycin **(50)** and it was reasoned that if two molecules were joined via a linker it might be possible to create a

new class of DNA bisintercalators **(48)** with greater biological activity. However biological testing *in vitro* showed that the bisintercalator **(48)** were less active than the simple anthracenedione **(49)** on leukaemia cell lines[43].

1.2.13 Anthracene-9,10-dione oligodeoxynucleotide conjugates

Oligodeoxynucleotides (ODN's) are short length of manmade genetic material[44] and are capable of binding to a DNA double helix which results in the formation of a triple helix[45]. ODN's are designed to specifically target genes and interfere with protein production[46]. One problem with ODNs is their poor cellular uptake[46] so they are combined with other intercalating molecules such as anthracene-9,10-dione. The combination of ODN's with anthracene-9,10-dione leads to derivatives that can specifically target cancerous cells rather than all rapidly dividing cells (see figure 1-5).

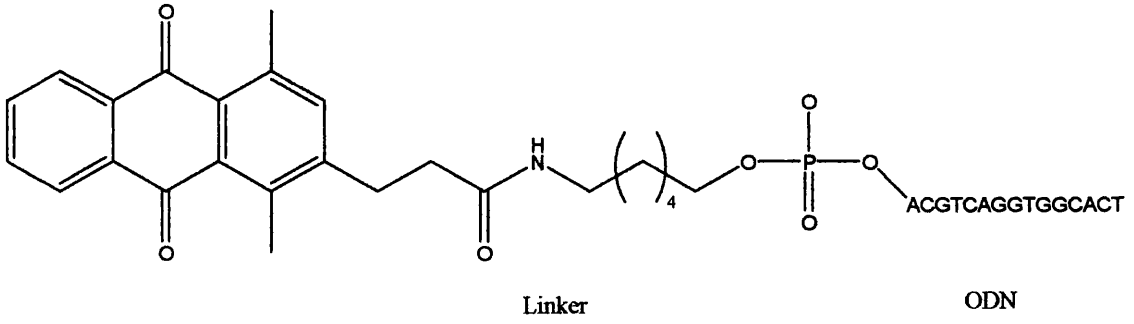


Figure 1-5. 1,4-dimethylanthracene-9,10-dione-oligodeoxynucleotide conjugate[47]

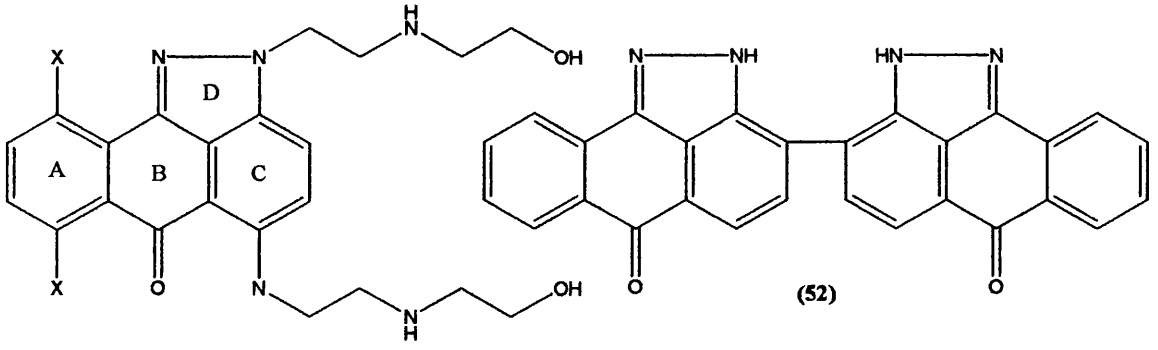
1.3 Hetero-substituted anthracene-9,10-dione analogues

The study of congeners with heteroatoms in the anthracene-9,10-dione nucleus is a relatively unexplored area. Heterocyclic analogues could potentially retain the same spatial and planar characteristics for DNA interaction but could also have extra bonding

or basic or reactive sites, and potentially have improved activity, and reduced cardiotoxicity.

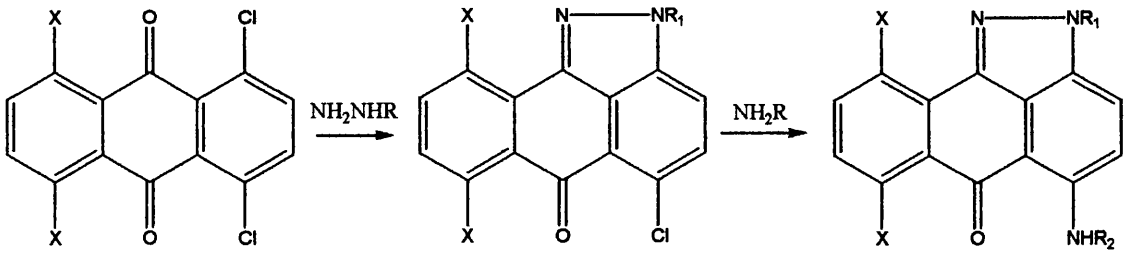
1.3.1 Anthrapyrazoles

The first reported synthesis of anthrapyrazoles **(51)** was in 1912 in conjunction with the dyestuff Pyrazoloanthrone yellow **(52)**[48].



(51)
(a) X= H
(b) X= OH

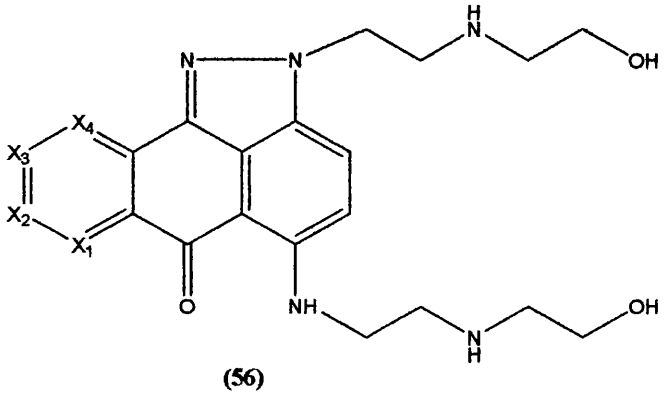
The quasi-iminoquinone considerably reduces ability to under go electrochemical reduction, in theory making the compound less cardiotoxic relative to Adriamycin[49]. Compounds **(51a-b)** were shown to have a unique biochemistry and an excellent pre-clinical *in vivo* anticancer activity and were entered into clinical trials[50]. Clinical trials showed good activity against breast cancer but as with other anthracenediones they showed cardiotoxicity[51]. The compounds are synthesised by a two-stage condensation, starting with the requisite 1,4-dichloro-anthracene-9,10-dione precursors **(53)**.



(53) (54) (55)

X=H or OH

Further reported developments include the synthesis of the aza-anthrapyrazole. It is interesting to note that the position of the nitrogen in the ring system is of importance and only 9-aza-anthrapyrazole **(56b)** is active[52].



(56)
(a) X_1=N X_2=X_3=X_4=C
(b) X2=N X3=X4=X1=C
(c) X3=N X4=X1=X2=C
(d) X4=N X1=X2=X3=C

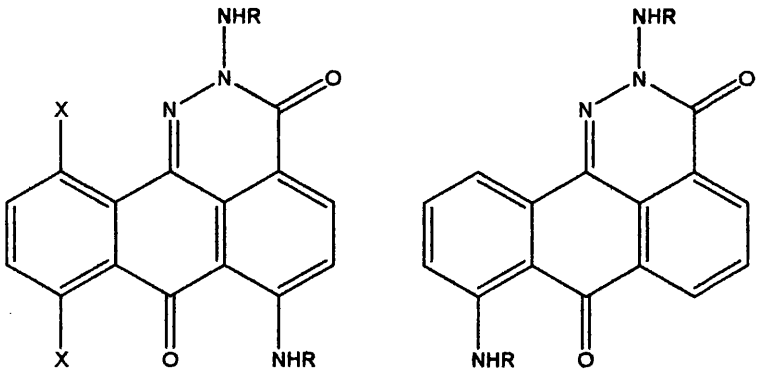
Anthrapyrazoles represent an important development in the anthracenedione class of drugs as they show activity against cancer cells lines that are resistant to other drugs like Adriamycin, Daunomycin, Mitoxantrone Amentantrone, etc. The prolonged treatment of cancer patients with drugs has led to the development of tumours that are resistant to the drugs and is the major reason why chemotherapy fails. Multi-drug resistance (MDR), is the principal reason many cancers develop resistance to antineoplastic agents. Tumours usually consist of mixed populations of malignant cells, some of which are drug-sensitive

while others are drug-resistant. Chemotherapy kills drug-sensitive cells, but leaves behind a higher proportion of drug-resistant cells. As the tumour begins to grow again, chemotherapy may fail because the remaining tumour cells are now resistant[53]. MDR is due to the presence of at least two molecular "pumps" in tumour-cell membranes that actively expel chemotherapy drugs from the interior thus rendering them useless. These pumps include P-glycoprotein and the so-called multi drug resistance–associated protein (MRP). This has led to a search for drugs, which can combat MDR.

One benefit from the discovery of MDR is it can be used to protect chemotherapy-sensitive non-cancerous cells, such as bone marrow stem cells, which need to be protected from the effects of chemotherapeutic agents. Bone marrow destruction is the single most important dose-limiting toxicity factor in the treatment of cancer patients. One reason is that recovery of bone marrow requires the removal of the patient from the chemotherapy regime, thus allowing cancer cells to grow again. If MDR can be conferred to such cells, patients could be given higher doses of anticancer agents than could be given normally.

1.3.2 Anthrapyridazones[54]

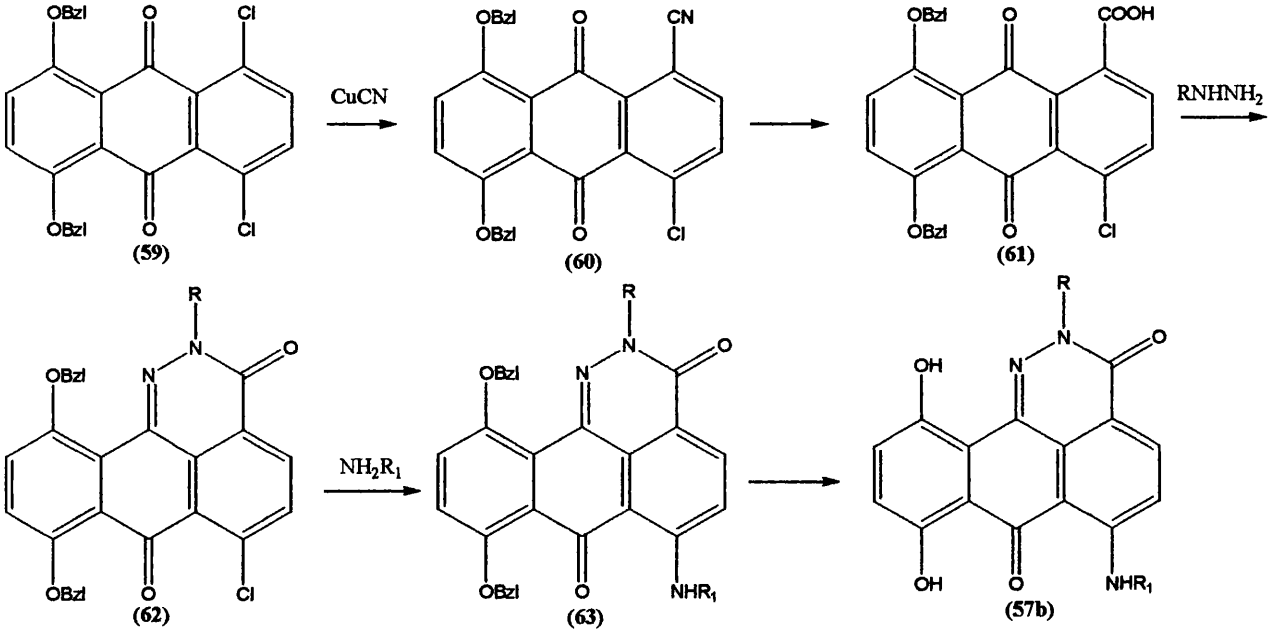
Following the success of the anthrapyrazoles it was postulated that the presence of a heterocyclic ring condensed with the anthracenedione chromophore could produce compounds with cytotoxic activity towards multi drug resistant cells. Anthrapyridazones **(57, 58)** represent such a class of compounds.



(57)
(a) X=H
(b) X=OH

(58)

As previously found with other anthracenediones the presence of hydroxyl groups on the chromophore greatly increases the anti tumour activity **(57b)**. Synthesis is achieved via the suitably protected 1,4-dichloro-5,8-dihydroxyanthracene-9,10-dione **(59)**.

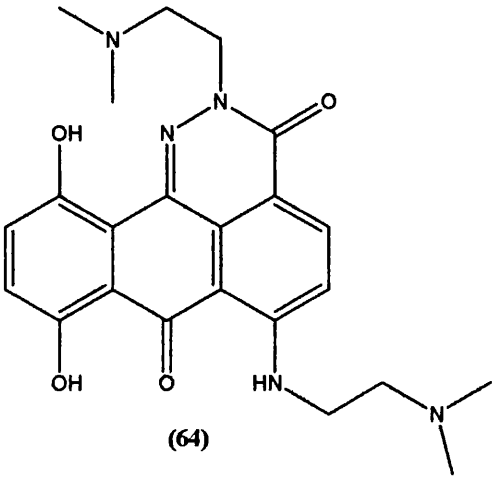


(59) (60) (61)

(62) (63) (57b)

Scheme 1-5. Synthesis of Anthrapyridazones

The anthrapyridazones have been evaluated for cytotoxicity. The 2,6 disubstituted derivatives **(57b)** are more active than 6-mono substituted derivatives **(58)**. Compound **(64)** in particular has shown increased activity over Mitoxantrone in MDR cell lines.

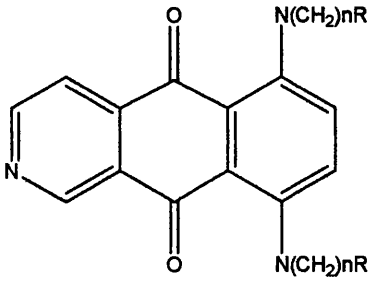


(64)

The anthrapyridazones constitute a novel class of anthracenedione analogues capable of overcoming multi drug resistance of tumour cells due to the presence of the heterocyclic ring system.

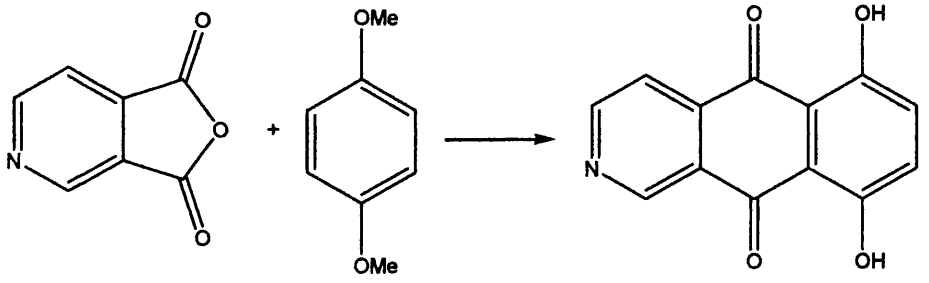
1.3.3 6,9-Bis[(amino alkyl)amino]benzo[g]isoquinoline-5,10-diones

In a search for anthracene-9,10-dione analogues with increased efficiency it was suggested that nitrogen atoms in the ring, such as structure **(65)**, might have increased affinity for DNA and improve the ability of the chromophore to intercalate[55].



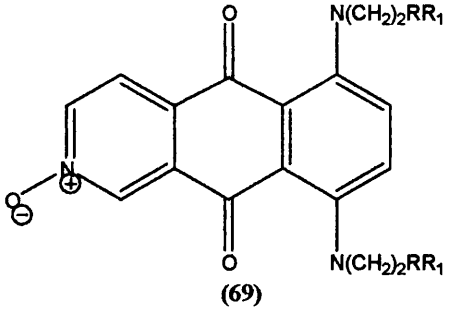
(65)

Synthesis is achieved via condensation of pyridine-3,4-dicarboxylic anhydride **(66)** with 1,4-dimethoxybenzene **(67)**, followed by the reduction of the product **(68)** and subsequent amination.



(66) (67) (68)

This group of compounds exhibits a wide range of *in vitro* activity, including drug resistant cell lines. Activity is also displayed in a N-oxide derivative **(69)**



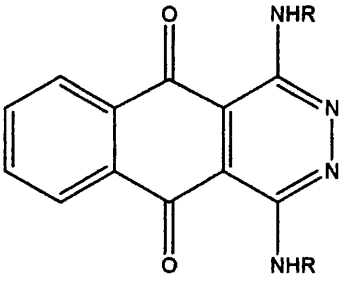
(69)

$R=R_1=CH_3$ or H

These heterocyclic bioisosteric models are representative of the first anthracene-9-10-diones, which display antileukemic activity comparable to Mitoxantrone.

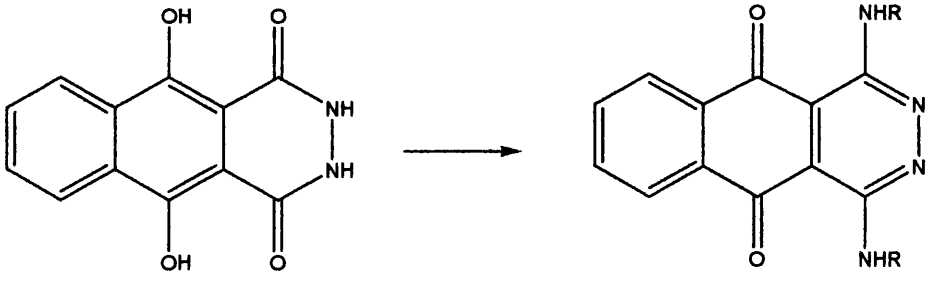
1.3.4 1,4-Bis[(amino alkyl)amino]benzo[g]-phthalazine-5,10diones

To further research on 6,9-bis[(aminoalkyl)amino]benzo[g]isoquinoline-5,10-diones the 2,3-diaza group was introduced into the anthracene-9,10-dione nucleus **(70)**.



(70)

Synthesis was attempted via oxidation of the appropriately protected anthracene but yields were poor. The reaction however was achieved via a one step silyliation-amination of the appropriately reduced quinone **(71)**.



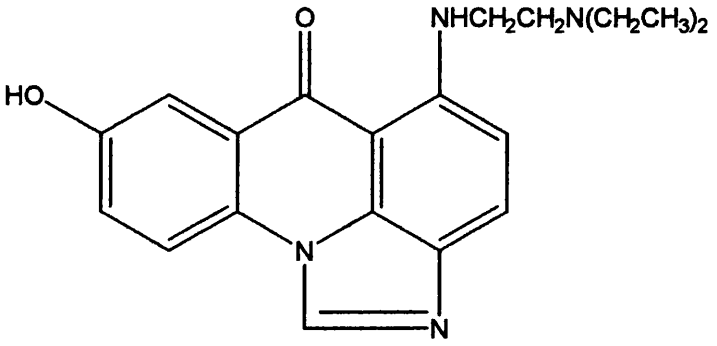
(71) **(70)**

Scheme 1-6. Synthesis of 1,4-bis[(amino alkyl)amino]benzo[g]-phthalazine-5,10-diones

The introduction of the diaza group into the anthracene-9,10-dione nucleus reduces the affinity for DNA compared to Amentantrone but does produce compounds with a high level of activity *in vitro* but there is no *in vivo* activity [56].

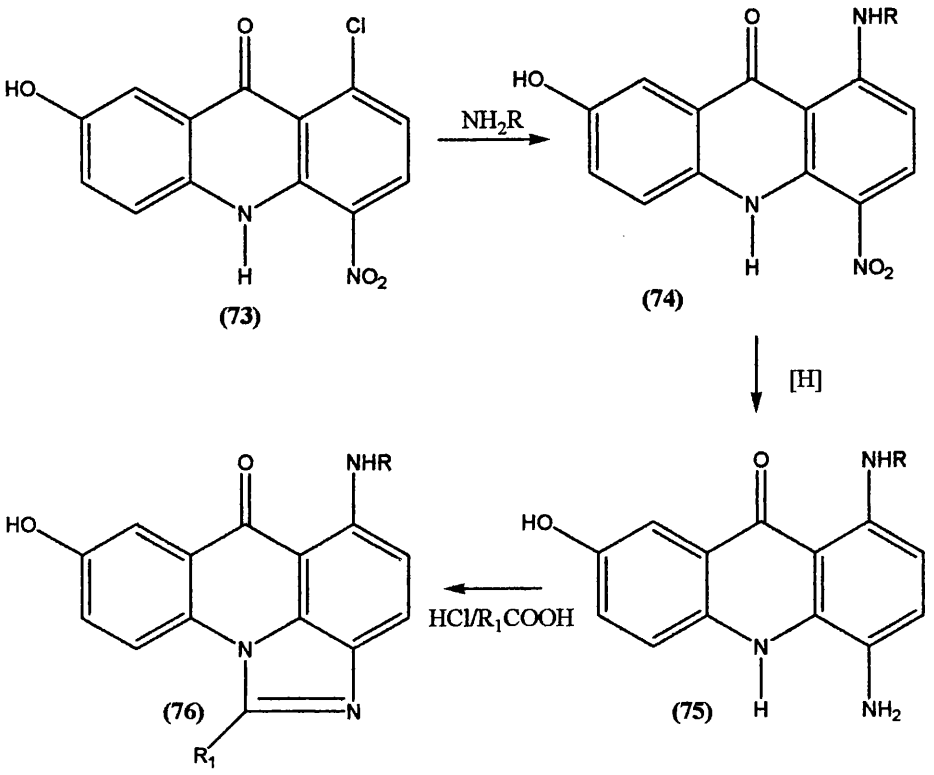
1.3.5 Imidazoacridinone derivatives

This group of compounds (**72**) is highly reactive *in vitro* and *in vivo* against tumour cell lines. It enters cells rapidly and accumulates within the nucleus. They are currently under going phase I clinical trials[57].



(72)

Synthesis is achieved from amination of 1-chloro-7-hydroxy(methoxy)-4-nitro-9(10H)-acridone (**73**) to give (**74**). The nitro group of (**75**) is then reduced and reacted with the appropriate carboxylic acid.

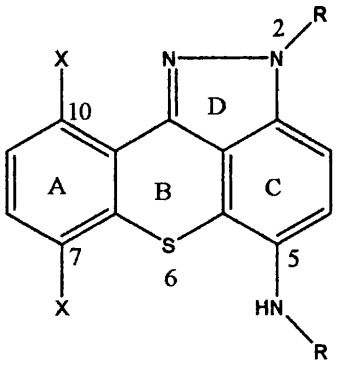


Scheme 1-7. Synthesis of imidazoacridinone derivatives.

Studies have show that molecules with the hydroxyl group in the same position of **(72)** are much more active though the aminoalkyl side chain is essential for high activity, especially the diethylaminoethylamino side chain. These derivatives inhibit catalytic activity of topoisomerase. They also induce the arrest of the cell cycle and cause apoptosis. The mechanism is not fully understood, though metabolic activation is considered as a preliminary step for their biochemical action. The metabolically activated drug is probably more reactive towards nucleophilic agents such as pyrimidine bases in DNA or sulfhydryl groups in proteins.

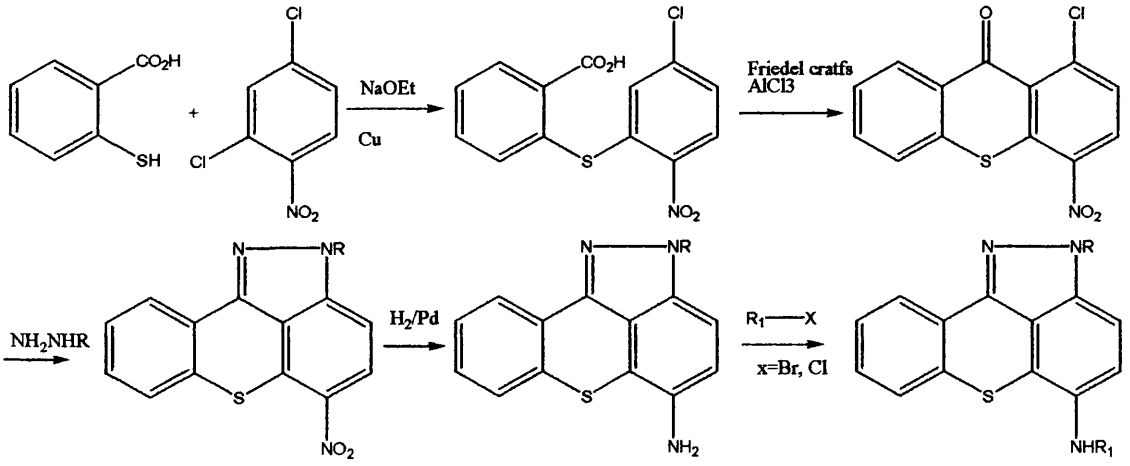
1.3.6 Benzothiopyranoindones

This class of compounds are related to the anthrapyrazoles, but a sulphur atom has replaced one of the carbonyl groups **(77a,b)**.



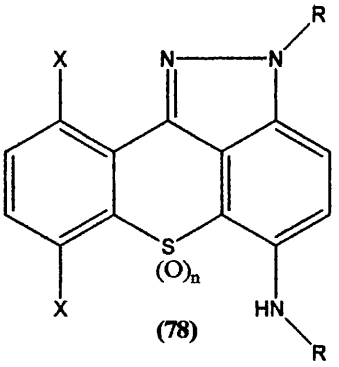
(77)
(a) X=OH
(b) X=H

The substitution of the sulphur atom virtually eliminates the possibility of redox cycling and subsequent radical formation *in vivo*. Synthesis is achieved via reaction of thiosalicylic acid with 2,4-dichloronitrobenzene (Scheme 1-8).



Scheme 1-8. Synthesis of benzothiopyranoindones

Some compounds showed good activity *in vivo*. Also synthesised were the corresponding oxides of sulphur **(78)**.



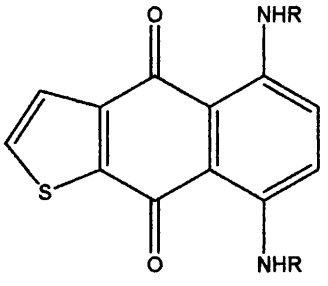
(78)

n=1 or 2

However, the higher oxidation state on the sulphur atom in the molecule had a deleterious effect, and reduced activity. This can be attributed to both electronic and steric effects, resulting in a lower electron density in the chromophore and loss of the planar nature of the chromophore, with the latter being the most detrimental as it prevents effective DNA intercalation.

1.3.7 5,8-Bis[(amino alkyl)amino]naptho[2,3-b]thiophene-4,9-diones

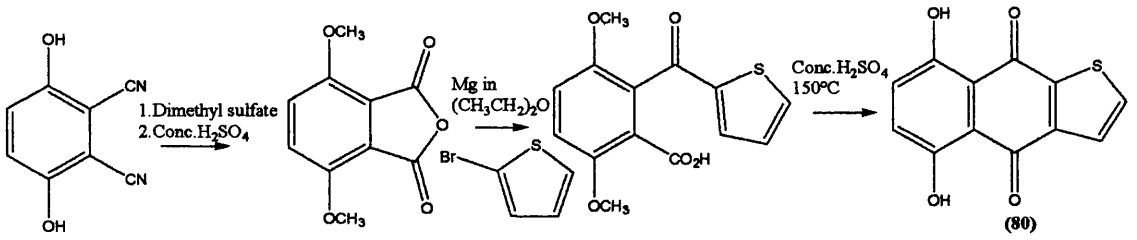
In a further attempt to introduce sulphur into the nucleus one of the benzyl rings of the anthracenedione molecule was replaced with a thiophene ring **(79)**[58]



(79)

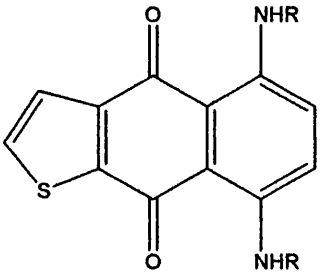
These thiophene analogues should maintain the planarity and spatial electronic characteristics of the carbocyclic quinone model that may be necessary for molecular

recognition at a cellular level (e.g. intercalation). They are prepared from commercially available 2,3-dicyanohydroquinone as shown (Scheme 1-9).



Scheme 1-9. Synthesis of 5,8-bis[(amino alkyl)amino]naptho[2,3-b]thiophene-4,9-diones

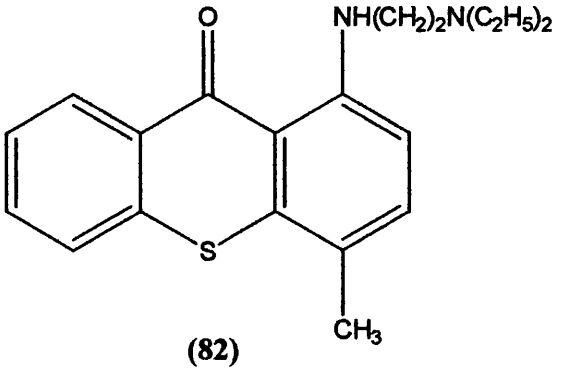
Intermediate **(80)** is then deprotected and reduced to form the leuco anthracenedione, which is then reacted with the appropriate amine. Biological activity of compounds **(81a,b),** is comparable to Mitoxantrone on leukemic cells.



(81)
(a) R= $(CH_2)_2NH_2$
(b) R= $(CH_2)_2NH(CH_2)_2OH$

1.3.8 Thioxanthone

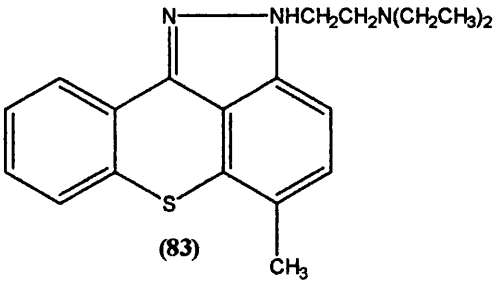
During the second world war German scientists developed a drug known as Miracil D or Thioxanthone **(82)**, which is a orally administered drug for the treatment of Schistosomiasis, also known as bilharziasis, a parasitic disease that leads to chronic ill health.[59]



(82)

Miracil D is prepared from condensing diethylaminoethylamine with 1-chloro-4-methylthiaxanthone, which is obtained from the reaction of thiosalicylic acid with *p*-chlorotoluene in sulphuric acid. Although this generates a mixture of 2 isomers, the 1-chloro derivative is sufficiently reactive to selectively condense with the amine[60]. It is interesting to note that derivatives with maximum activity have a separation of two carbons between the two nitrogen's in the side chain, which is analogous to the activity shown by Mitoxantrone. Miracil D also shows some anti-tumour activity. In attempt to improve the activity further the side chain was replaced with the Mitoxantrone aminoalkyl side chain. This increased the activity but not to a level with any therapeutic potential[61].

Recent research has show that Miracil D or Luctathone as it is also known can be used as radio sensitising agent in cancer radiotherapy. It specifically targets topoisomerase II, trapping the enzyme and DNA in a complex. This research has led to the synthesis of the analogue **(83).**

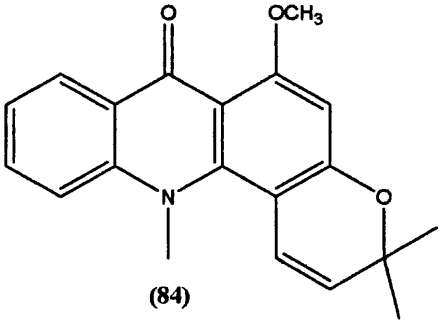


(83)

This compound is very closer related to the benzothiopyranoindones (Section 1.3.5.). It has shown good activity *in vitro*.[62]

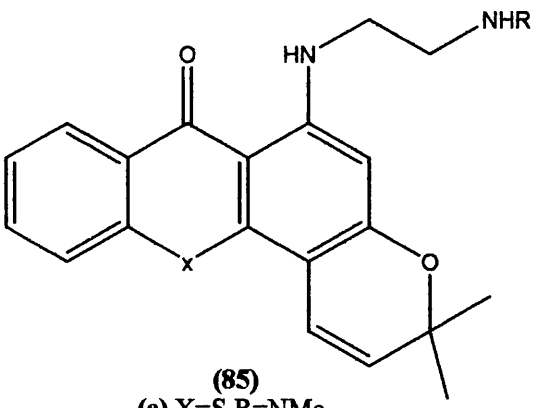
1.3.9 Acridones

Acronycine **(84)** the acridone alkaloid isolated from *Acronychia baueri (Rutaceae)*[63] was found to have *in vivo* activity against tumours.

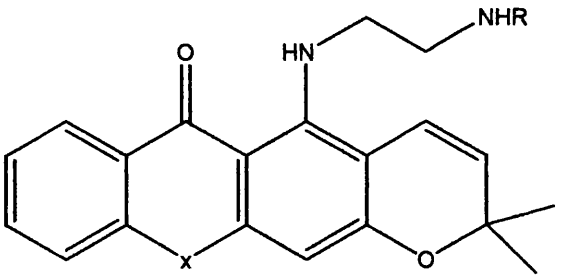


(84)

However due to it poor water solubility it was unsuitable for clinical use. Compounds **(85-86)** were synthesised in an attempt to combine some of the features of acronycine and thioxanthone, also synthesised were some oxygen analogous.



(85)
(a) X=S R=NMe_2
(b) X=O R=NMe_2
(c) X=S R=NEt_2
(d) X=O R=NEt_2

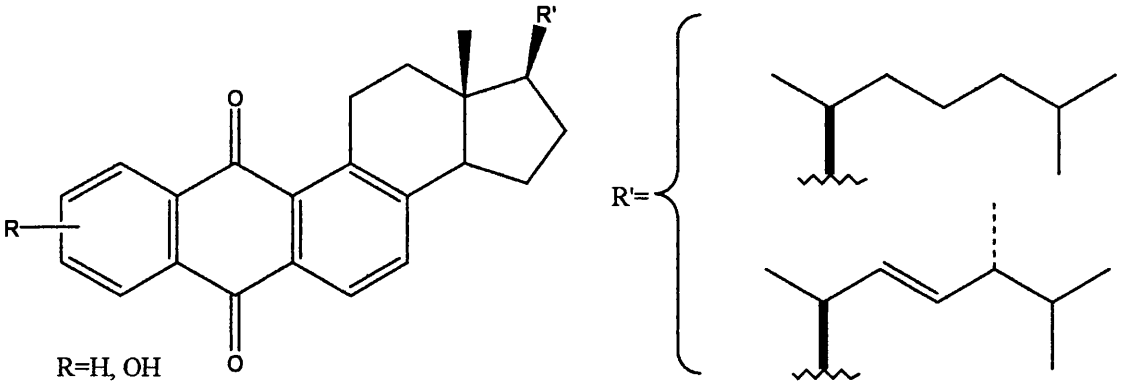


(86)
(a) X=S R=NMe_2
(b) X=O R=NMe_2
(c) X=S R=NEt_2
(d) X=O R=NEt_2

In general all derivatives are more active than acridone but are still not as active as Mitoxantrone.

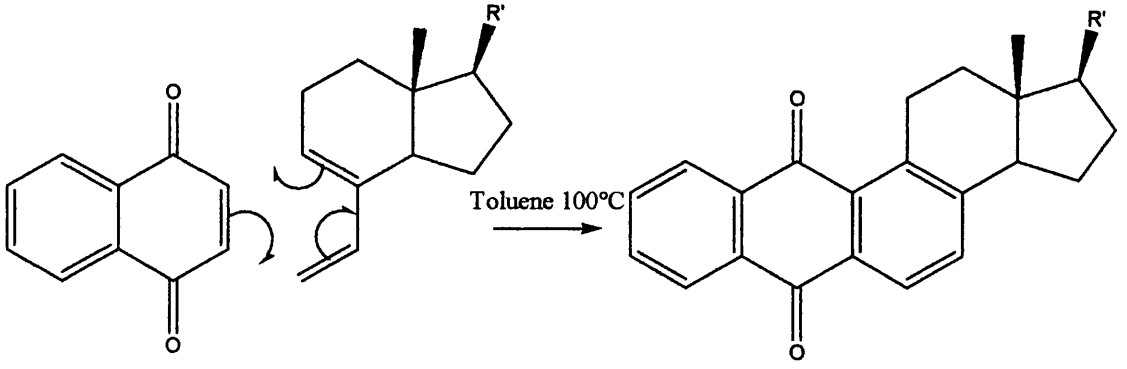
1.3.10 Steroid-Anthracene-9,10-dione Hybrids

The combination of different classes of bioactive compounds can produce novel and potentially bioactive compounds. Taking into account the effectiveness of anthracene-9,10-dione based drugs in the field of chemotherapy the combination of the anthracene-9,10-dione molecule with steroids, which are important vectors for biological activity, could lead to drugs with enhanced activity, such as **(87).**



(87)

The steroid-anthracene-9,10-dione hybrids were synthesised using a Diels-Alder approach, (Scheme 1-10).

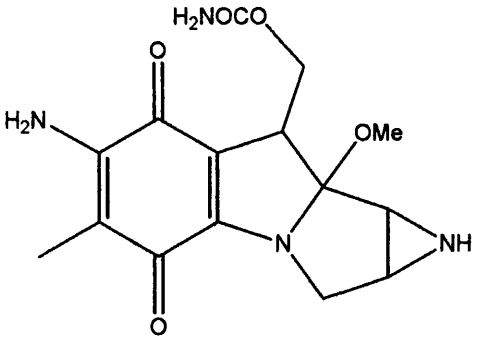


Scheme 1-10. Synthesis of steroid-anthracene-9,10-dione hybrids

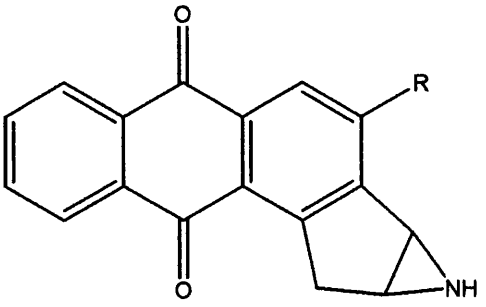
Initial testing has shown some derivatives to be as active as doxorubicin *in vitro* and further investigations are in progress to asses the full potential of these compounds.[64]

1.3.11 Cyclopent[a]anthracene-9,10-dione derivatives

MMC **(88)** (mitomycin C), is a bioreductive-alkylating agent that cross links DNA and inhibits cancerous cells. However it also destroys healthy cells, as it is not selective enough for clinical use. In an attempt to improve selectivity, anthracene-9,10-dione analogues of MMC were synthesised **(89a-b)**.



(88)



(89)
(a) R=$OCH_2CH_2N(CH_2CH_2Cl)_2$
(b) R=$CH_2OCONHMe$

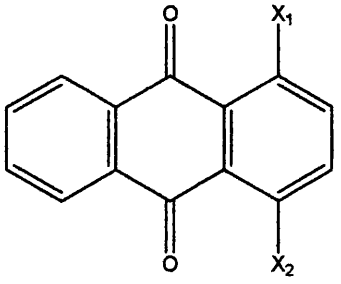
These analogues should have the ability to intercalate into DNA and bind covalently. Compound **(89a)** shows good *in vitro* activity against leukaemia cells, although it is less potent than **(88)**. The main draw back of compound **(89a)** is the life span, as it decomposes after 3 months due to the presence of the mustard side chain. As a result 2,3,-aziridino-4-[[(methylaminocarbonyl]methyl]cyclopent[a]anthracene-6,11,dione **(89b)** was synthesised and was found to be just as effective against leukemic and solid tumour cells as **(88)** *in vitro,* and it appears to be less toxic. It is also able to intercalate into DNA and function as a topoisomerase II inhibitor. Further testing is required to fully evaluate the potential of this class of anthracene-9,10-diones[65]

1.4 Other uses of anthracene-9,10-diones

1.4.1 Anthracene-9,10-dione dyes

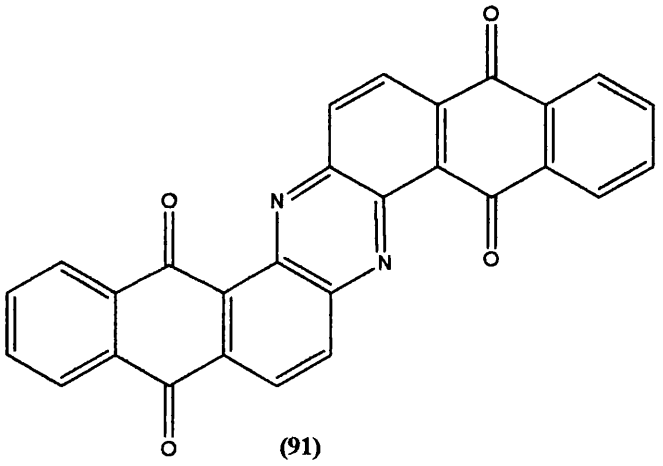
The main use of anthracene-9,10-dione has been in the manufacture of dyestuffs. Many derivatives have been synthesised and used as dyes for cotton, polyester, nylon, and wool. They can also be used as colorants for plastic, wood, and paints. Although anthracene-9,10-dione itself does not absorb in the visible region introduction of different

groups into one of the 8 positions, produces compounds that do absorb in the visible region [66] Anthracene-9,10-dione derivatives that possess substituents that are good electron donors have deeper and stronger colour **(90a-c)**



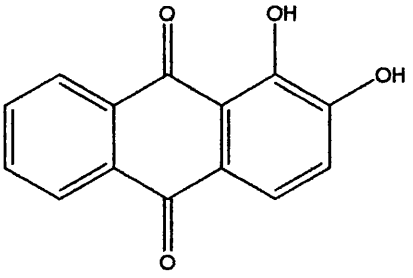
(90)	**Colour**
(a) X_1=H X_2=NO_2,Cl or Br	Yellow
(b) X_1=H X_2=NH_2,O, NHMe or NHPh	Orange to red
(c) X_1= X_2=NH_2,NHMe or NHPh	Blue

In 1901 Rene Bohn patented the first anthracene-9,10-dione vat dye Indanthrene blue RS or 6,15-dihydro-5,9,14,18-anthrazinetetrone [67]**(91)**.



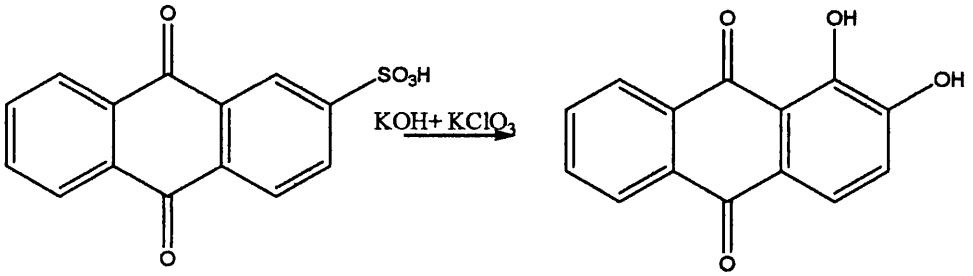
(91)

It is still in use today as a food colouring E130. Anthracene-9,10-dione dyes such as alizarin **(92)** are vat dyes, as they can be reduced to form more soluble compounds, and can then be oxidised once on the fabric. Alizarin **(92)** occurs naturally as a glycoside in the roots of madder, which was used by the ancient Egyptians as a dyestuff.



(92)

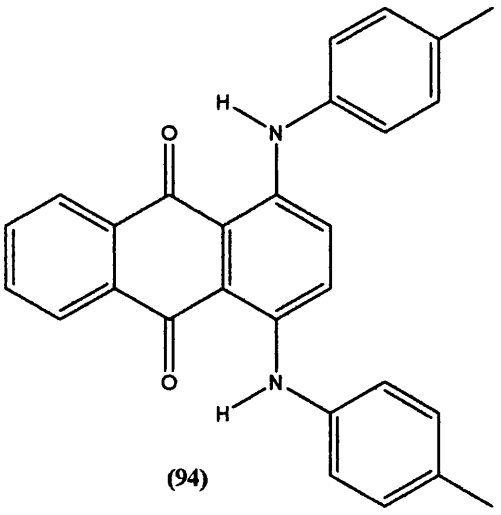
Alizarin dissolves in alkali giving purple or red solutions, which are precipitated, as lakes by heavy metal salts. It is now synthesised from anthracene-9,10-dione-2-sulphonic acid **(93)** with sodium hydroxide and $KClO_3$[68] (Scheme 1-11). Alizarin is an important starting material for other anthracene-9,10-dione dyes.



(93) (92)

Scheme 1-11. Alizarin synthesis

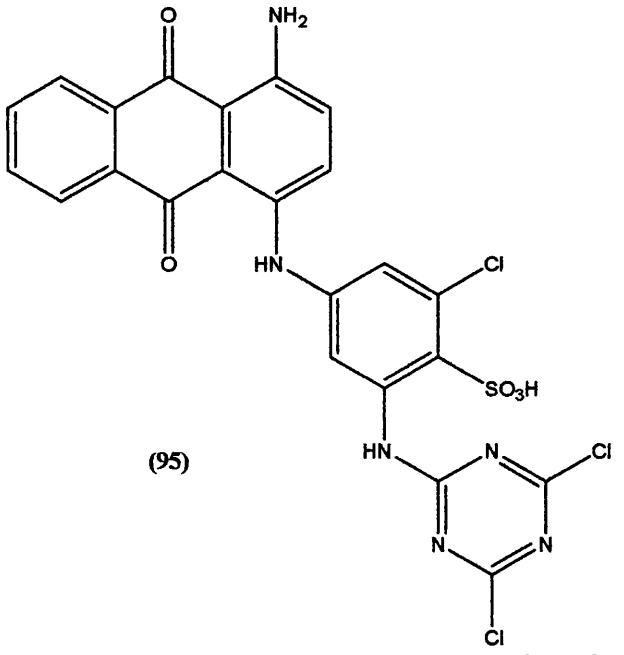
Aminoanthracene-9,10-diones include dyes such as Quinizarin Green (1,4-bis(p-toluidinio)anthracene-9,10-dione) **(94)** ,which is a disperse dye. Most anthracene-9,10-dione dyes are disperse or reactive dyes.



(94)

Disperse dyes have low solubility in water, but they can interact with the polyester chains by forming dispersed particles. Their main use is the dyeing of polyesters, and they find minor use dyeing cellulose acetates and polyamides. The dye is generally applied under pressure, at temperatures of about 130°C. At this temperature, thermal agitation causes the polymer's structure to become looser and less crystalline, opening gaps for the dye molecules to enter. The interactions between dye and polymer are thought to be Van-der-Waals and dipole forces.[69]

Anthracene-9,10-dione reactive dyes are the second most important type of reactive dyes. Most of these types are derived from Bromamine acid (1-amino-4-bromoanthracene-9,10-dione-2-sulphonic acid) and by variation of the substituents, a wide range of colours can be obtained, such as Reactive Blue Seven (**95**).

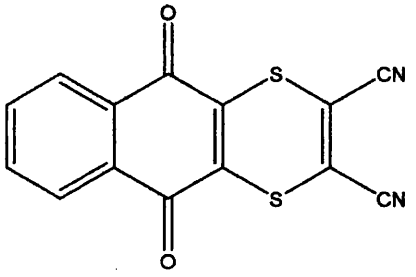


(95)

There are three steps in the reactive dyeing procedure, adsorption of dye on the fibre, diffusion into the fibre and the covalent reaction with the particular nucleophilic groups present in the substrate[70].

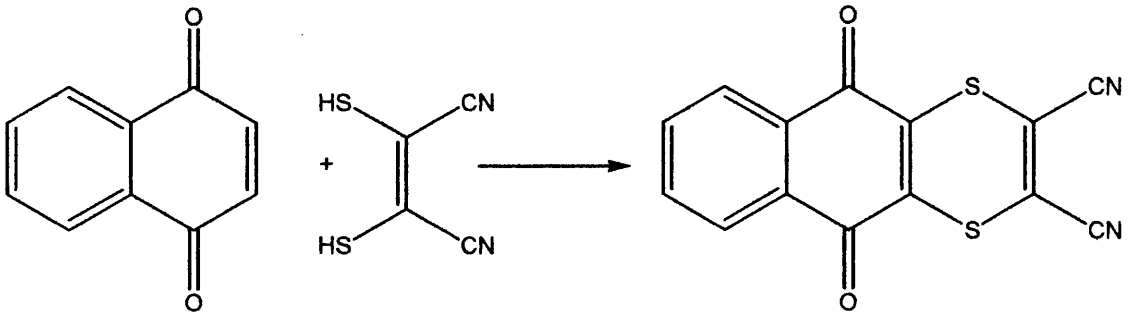
1.4.2 Dithianon

Dithianon or 1,4-dithianthracene-9,10-dione **(96)** (5,10-dihydro-5,10-dioxo-naphto-[2,3]-p-dithio-2,3-dicaronitrile) is a protective and nonphytotoxic fungicide with a broad-spectrum activity for the control of diseases of pomes and stone fruit, small fruit, citrus, coffee and wine growing[71].



(96)

E.Merck introduced Dithianon in 1962 under the trade name Delan®. It forms odourless crystals, which melt at 225°C. It is produced from the reaction of the sodium salt of 1,2-dicyano-1,2-dimercaptoethylene and napthaquinone[72] (Scheme 1-12).



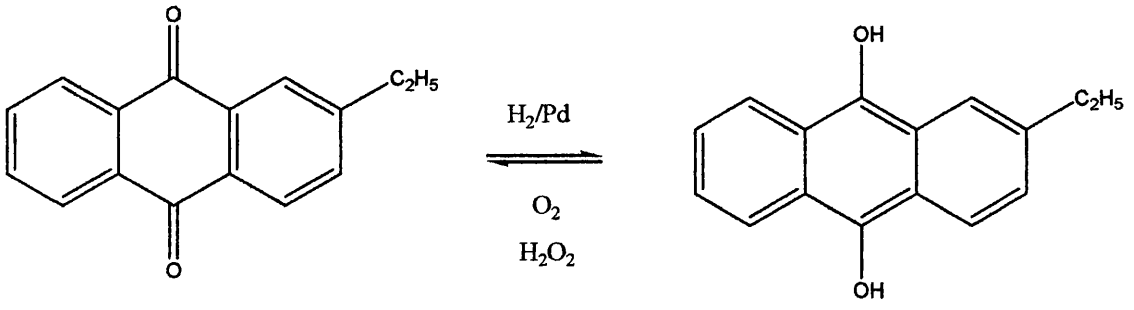
(97) (98) (96)

Scheme 1-12. Synthesis of dithianon.

It is insoluble in water but soluble in dioxanes, chlorobenzene and chloroform. The compound is stable in weak acid and neutral media. In alkaline conditions ring opening occurs.

1.4.3 Hydrogen peroxide Synthesis

2-Ethylanthracene-9,10-dione **(99)** is used in the commercial production of hydrogen peroxide. By an initial reduction with hydrogen and palladium to 2-ethylanthraquinol **(100)**. Oxygen is then bubbled through the solution to oxidize the reduced molecule back to 2-ethylanthracene-9,10-dione **(99)** with the subsequent production of hydrogen peroxide (Scheme 1-13).



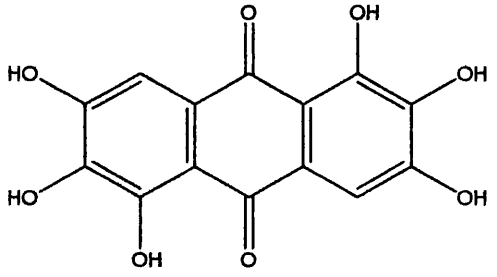
(99) (100)

Scheme 1-13. Synthesis of hydrogen peroxide with anthracene-9,10-dione

This process is much cheaper than the electrochemical production of hydrogen peroxide.

1.4.4 Laxatives

Anthracene-9,10-diones have also been used as laxatives, for example the polyhydroxy derivatives such as Rufigallic or 1,2,3,5,6,7-hexahydroxyanthracene-9,10-dione **(101)**, an abstract of rhubarb has been used in Chinese herbal medicine since 2400 BC[73].



(101)

1.4.5 Inhibition of HIV-1 proteinase[74]

HIV-1 proteinase is a key enzyme in the replication of the HIV virus, which causes the disease, AIDS. Several inhibitors have been developed some, which compete for the substrate-binding grove of the enzymes. The most effective inhibitors have one or more hydroxyl substituents and are usually peptides. To develop non-peptide drugs the

hydroxy-quinones were chosen for test for proteinase inhibition, it was found that the anthracene-9,10-dione derivatives were very effective, alizarin **(92)** in particular. Hydroxyanthracene-9,10-diones might therefore be a useful component in the development of a potent and selective drug against HIV viruses.

1.4.6 Multiple sclerosis

Multiple sclerosis is considered an autoimmune disease associated with immune activity directed against the central nervous system. Based on this concept immunosupression has been the therapeutic strategy to combat the disease, which has lead to the development of understanding and treatment of the disease. Mitoxantrone has shown to be effective for active multiple sclerosis because of its ability to depress the immune system. The only real problem with the drug is again its cumulative cardiotoxicity[75].

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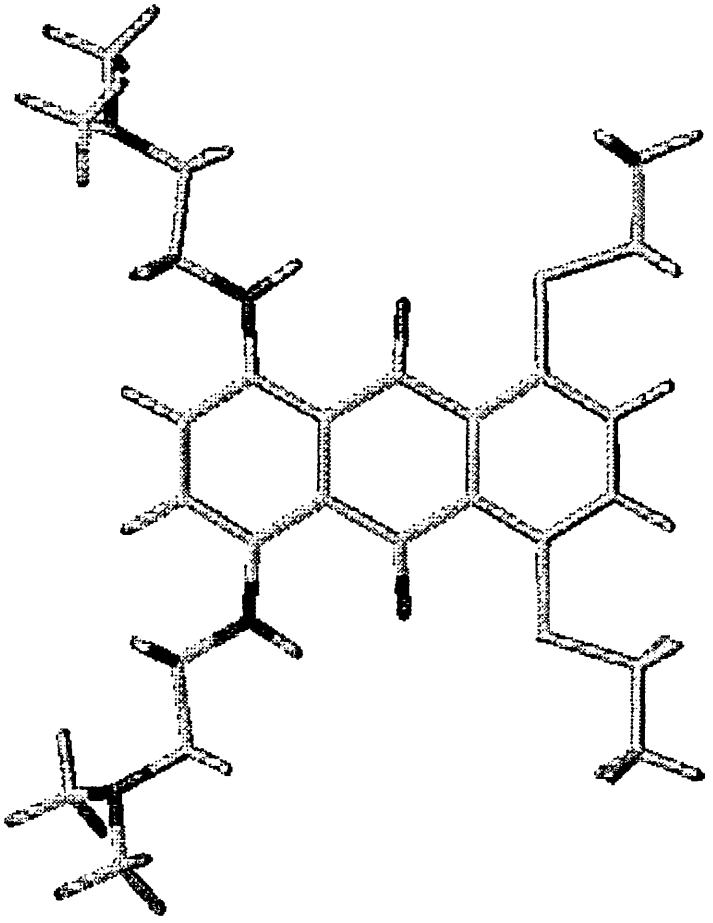
CHAPTER TWO



AIMS & OBJECTIVES

2.0 Aims and objectives

Anthracene-9,10-dione forms a wide and important class of dyes though they have by and large, been superseded by Azo dyes which are cheaper and easier to produce. However, there has been renewed interest in anthracene-9,10-dione due the discovery that some derivatives can be used in the treatment of cancer (such as the anthracycline Adriamycin and more recently some N-alkyl derivatives such as Mitoxantrone). The aim of this project was to synthesis some novel anthracene-9,10-dione type drugs containing sulfur and to explore their activity.

2.1 Effect of sulfur upon quinones

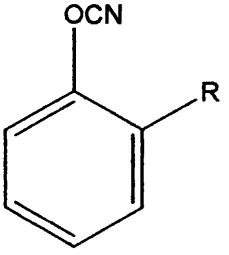
It is well documented that the addition of substituents to quinones leads to a significant effect on their redox potentials. In particular the addition of thiols to quinones has been the subject of a number of studies, as thioether formation is an important process in the detoxification and excretion of potentially toxic electrophiles within biological system. The addition of thiols to quinones can lead to an increase or decrease in the oxidation potentials[1]. This could lead to Mitoxantrone derivatives with reduced cardiotoxicity, as the one electron reduction of Mitoxantrone leads to free radicals that can then go on to damage heart tissue (see section 1.1.4 part II)

While the insertion of thiols to some quinones has led to deactivation of the biological activity, in some cases it has been shown that quinone-thioethers possess new biological activity and toxicological properties. There is substantial evidence supporting the interaction of quinone-thioethers with enzymes that have either quinones as substrates or with enzymes that have either quinones as substrates or with enzymes that utilize

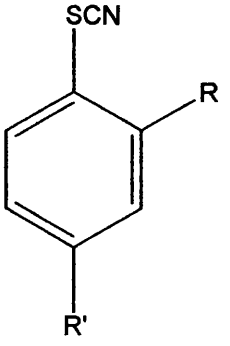
glutathione. Quinone-thioethers have been show to interact with DNA and have also been identified as compounds that play important role in various physical and behavioral changes that accompany chronic alcoholism[1].

2.2 Sulfur biocides

The ability of sulfur to interact within a biological systems, is illustrated by the chemistry of phenylcyanate **(102)** and phenylthiocyanate **(103)**. Neither unsubstituted compound **(102a, 103a)** displays any sign of biological activity though by the insertion of a nitro group at the 2 position of the benzene ring, results in a remarkable increase in efficacy of the phenylthiocynate **(103b)**[2]. In fact, such is the increase in biocidal nature that the resulting molecules have been patented as antivirals, antifungal, and antibacterials[3], in particular the 2,4-dinitrophenyl thiocyanate derivative **(103c)** discovered by Dupont. The 2-nitrophenylcyanate **(102b)** on the other hand remains relatively inactive leading to the conclusion that the sulfur atom appears essential for biological action.



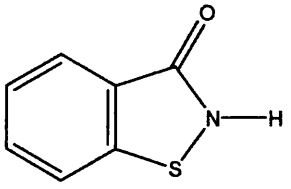
(102)
(a) R= H
(b) R=NO_2



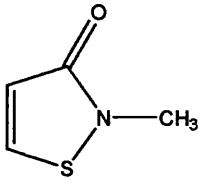
(103)
(a) R=R'= H
(b) R=NO_2,R'=H
(c)R=R'=NO_2

Some thiocyanates have also been reported to act as anti-tumour and anti-hormonal agents[2].

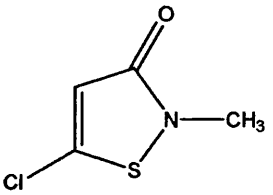
The reasons for the success and activity of sulfur containing compounds as biocides may be due to a number of factors. The ability of elemental sulfur to control pests and fungi has been known since 1000 BC and sulfur compounds have always been regarded as making good biocides. The capability of the sulfur atom to bond with the intracellular thiols is obviously a huge benefit as this and the resulting reactions *in vivo* may lead to the death of the cell. In other instances the organosulfur compounds will be broken down in a cell, yet the simple sulfur by-products e.g. H_2S, elemental S, or SO_2, are often just as toxic. Another group of important sulfur biocides include the isothiazolones of which some compounds possess unparalleled biocidal efficacy at low concentrations. The 4,5-benzo-3-isothiazolones (**104**) were first synthesized in 1923 by McKibben and McClelland[4] but the isothiazolones biocidal properties remained undiscovered until their anti-microbial properties were reported and patented in 1957 by Katz and Schroeder[5]. In 1973, the benzo-isothiazolones were joined in the biocide marketplace by substituted 3-isothiazolones which, as can be seen in the patent by Lewis[6], have a very broad spectrum of activity and yet are still active at extremely low concentrations. The Rohm and Haas product Kathon® contains a mixture of 5-chloro-*N*-methyl-3-isothiazolone (**105**) and *N*-methyl-3-isothiazolone (**106**) as its active ingredients.



(**104**)



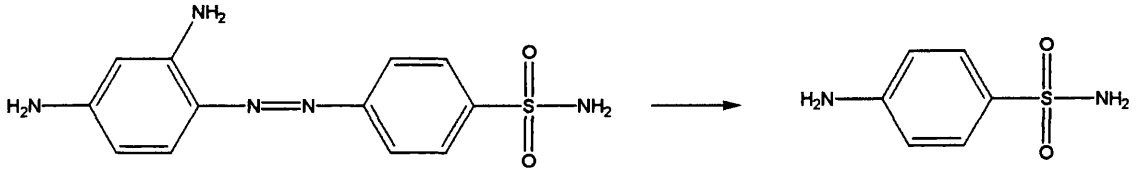
(**105**)



(**106**)

2.3 Sulpha drugs

Other important sulfur containing drugs include the Sulphonamides, also known as Sulpha drugs, which is common name applied to a group of chemotherapeutic agents that are effective against a number of infectious diseases. In 1935 the German chemist, Gerhard Johannes Paul Domagk, discovered that an azo dye, Prontosil **(107)**, cured streptococcal infections in mice. The active principle in Prontosil was found to be 4-aminobenzenesulphonamide **(108)**, commonly known as sulphanilamide[7], formed by the degradation of the azo linkage.



(107)

(108)

Scheme 2-1. The breakdown of Prontsil to give Sulfanilamide

Clinical trials with sulphanilamide proved effective in arresting various bacterial diseases. Many derivatives of sulphanilamide have proved effective against such conditions as scarlet fever, meningitis and pneumonia. All the sulphonamides are somewhat toxic, producing blood abnormalities and kidney damage when used indiscriminately. Since the discovery of penicillin, which is as effective as the sulphonamides although far less toxic, the use of sulphonamides has somewhat declined. Because bacteria often develop resistance to a particular kind of treatment, however, they are used when bacterial tolerance for penicillin has developed.

2.4 Conclusion

Sulfur is an important element within biological systems and is an essential dietary ingredient[8]. The introduction of sulfur into anthracene-9,10-dione drugs leads to a change in the electronic, steric and physical properties, which in turn effect how the molecule would interact with a biological system. The sulfur atom within in these drugs could also introduce another mode of action, as they may have the ability to undergo nucleophilic reaction with intracellular thiols. The present studies have been carried out therefore to synthesize a number of new anthracene-9,10-dione systems containing sulfur and to model their activity. It was planned to submit some of the derivatives for testing by AstraZeneca.

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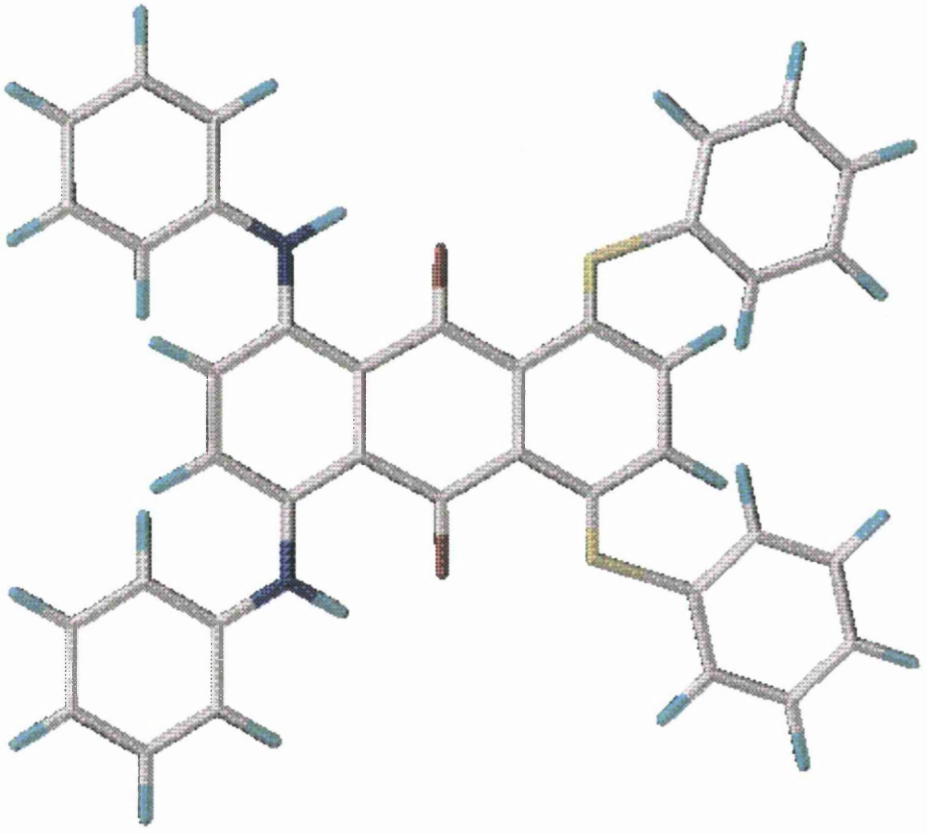
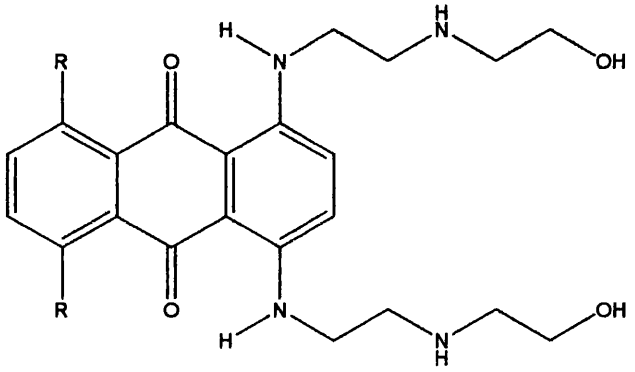
CHAPTER THREE



SYNTHESIS OF 1,4-BIS(AMINO)-5,8-(SULFANYL)ANTHRACENE-9,10-DIONES

3.0 Preparation of 1,4-bis(amino)-5,8-(sulfanyl)anthracene-9,10-diones

Both Amentantrone (24a) and Mitoxantrone (24b) are effective as anticancer drugs.

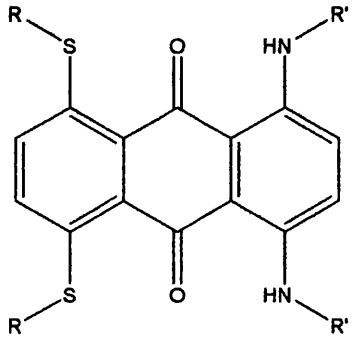


(24)
(a) R= H
(b) R= OH

As discussed earlier, these drugs are used clinically in the treatment of many cancers but they have associated side effects. As cardiotoxicity is the most serious side effect it is therefore the major dose limiting factor. Cardiotoxicity can be attributed to the ability of the anthracene-9,10-dione moiety to undergo a one electron reduction to form a radical species (see section **1.1.2.**).

The introduction of sulfur instead of oxygen at the 5- and 8- positions would lead to derivatives such as **(110)**, these 1,4-bis(amino)-5,8-(sulfanyl)anthracene-9,10-diones would have different electronic and steric properties to the parent drug which, could be potentially beneficial, i.e. a reduced ability to form free radicals or improved binding

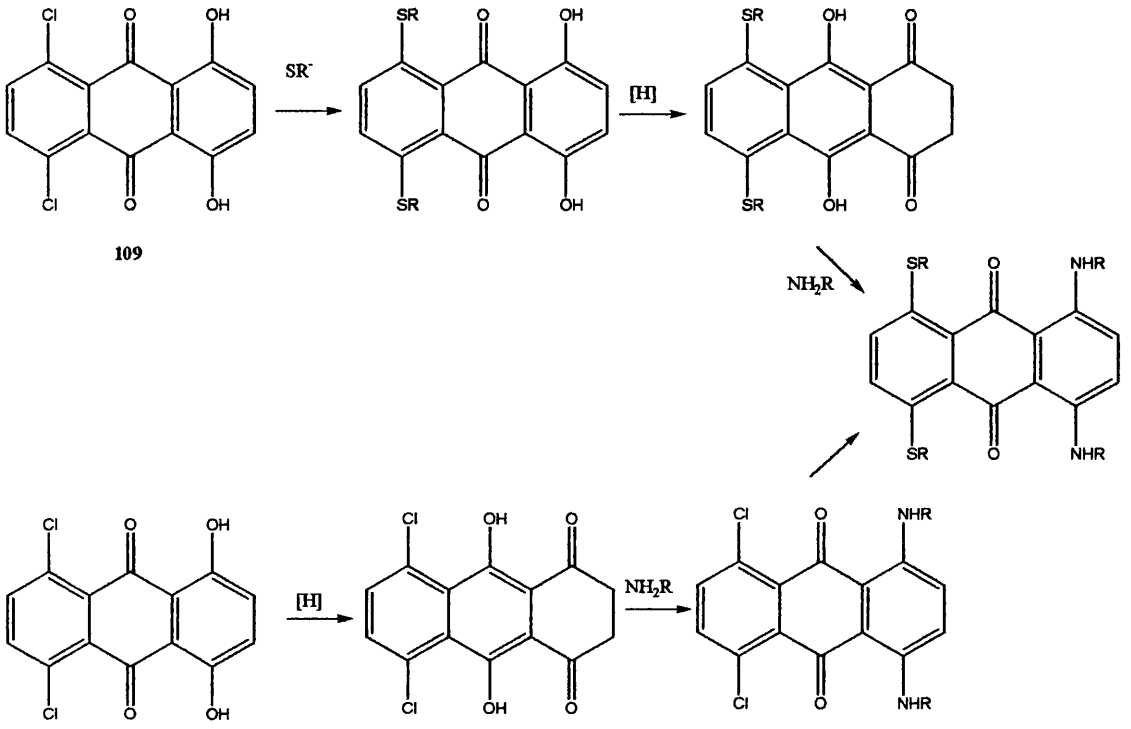
characteristics. The sulfur atom may also act as a reactive centre for important cellular thiols such as glutathione (see section 1.2.3).



(110)

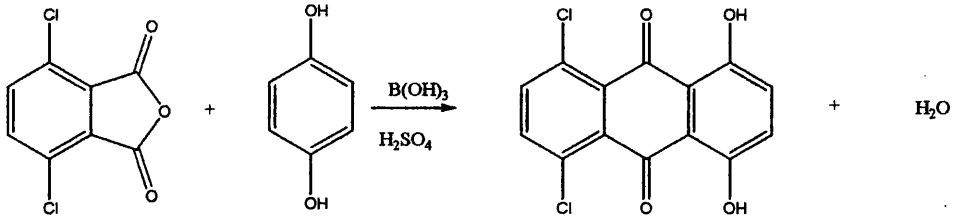
In principle the 1,4-bis(amino)-5,8-(sulfanyl)anthracene-9,10-diones **(110)** derivatives are obtainable from 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione **(109)** by two routes:

1. Displacement of the chlorine atoms of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione **(109)** with appropriately selected thiolate ions, followed by the subsequent reduction and amination with selected amines..
2. The reduction of 1,4-dihydroxyanthracene-9,10-dione **(109)**, which could then be aminated with the appropriate amines and thiolated, with selected thiols.



Scheme 3-1. Proposed synthetic routes

The starting material 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione can be synthesised from the condensation reaction of 3,6-dichlorophthalic anhydride with benzene-1,4-diol in concentrated sulphuric acid with boric acid[1].

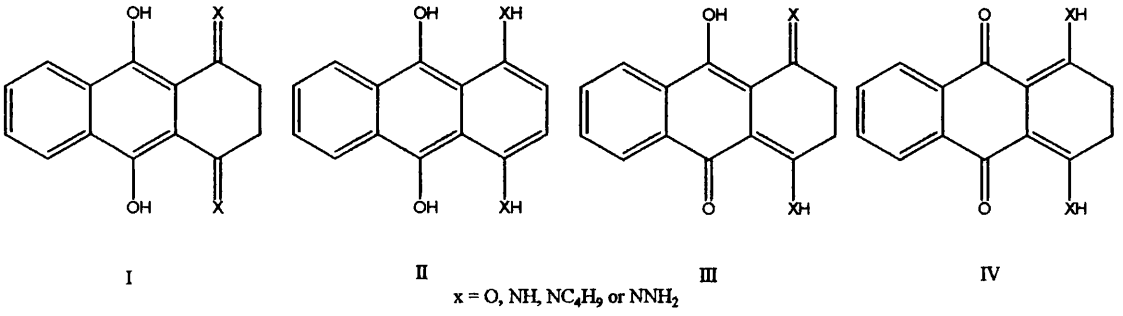


Scheme 3-2. Condensation of 3,6-dichlorophthalic anhydride with benzoquinone using boric acid in sulphuric acid.

Alternatively 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione can be synthesised by the direct chlorination of 1,4-dihydroxyanthracene-9,10-dione[2,3,4,5,6] (see section 3.2).

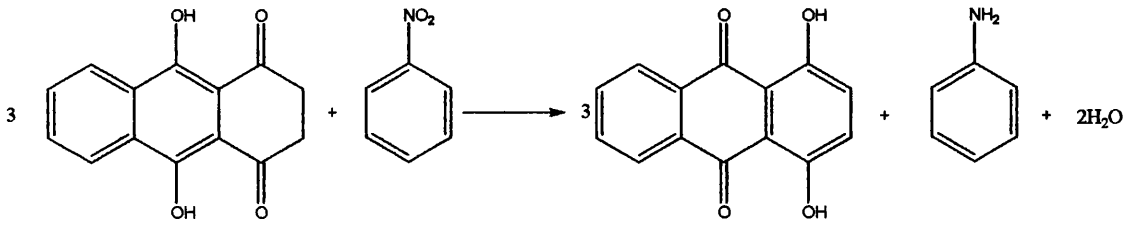
3.1 Oxidation of 2,3-dihydro-9,10-dihydroxyanthracene-1,4-dione

2,3-Dihydro-9,10-dihydroxyanthracene-1,4-dione **(110)** was used as a starting material as it was readily available from an industrial source (some 1,4-dihydroxyanthracene-9,10-dione was also purchased from Aldrich). It is the reduced or leuco form of 1,4-dihydroxyanthracene-9,10-dione (Quinizarin). It can exist in four possible tautomers (Scheme 3-3).



Scheme 3-3. Possible tautomers of leuco anthracene-9,10-diones

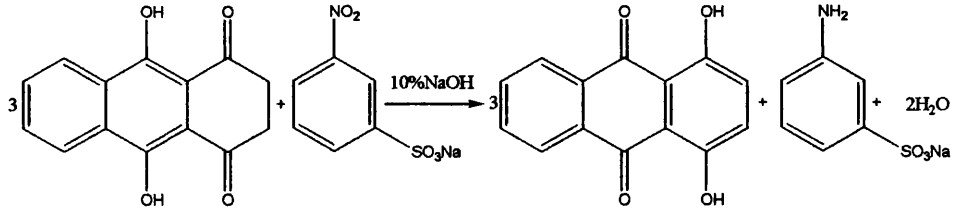
The leuco form of 1,4-dihydroxyanthracene-9,10-dione however occurs preferentially in only one tautomeric form 2,3-dihydro-9,10-dihydroxyanthracene-1,4-dione **(I)**[7]. The preparation of 1,4-dihydroxyanthracene-9,10-dione by oxidation of 2,3-dihydro-9,10-dihydroxyanthracene-1,4-dione **(110)** was achieved under reflux with nitrobenzene (Scheme 3-4) following the known industrial process[8].



(110) (111)

Scheme 3-4. Oxidation of 2,3-dihydro-9,10-dihydroxy-1,4-anthracene-9,10-dione using nitrobenzene

The major draw back of using nitrobenzene is the work up procedure, due to its high boiling point it needs to be removed via steam distillation for product recovery. A much more convenient method of oxidation involves the use of 3-nitrobenzenesulfonic acid sodium salt under alkaline conditions using water as the solvent[9]. The mixture is then heated to reflux and the product is simply collected by acidification of the mixture.



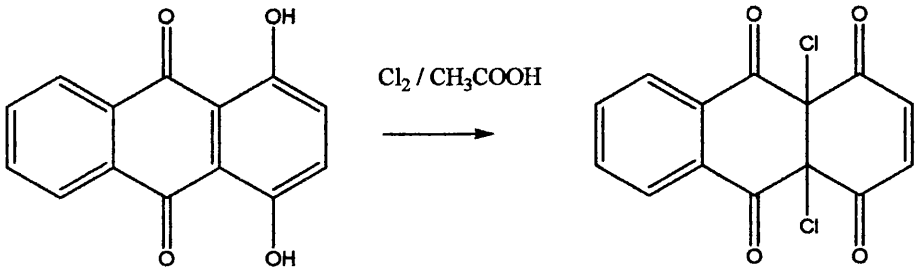
(110) (111)

Scheme 3-5. The oxidation of 2,3-dihydro-9,10-dihydroxy-1,4-anthracene-9,10-dione using sodium 3-nitrobenzenesulfonate

Oxidation was also achieved using activated manganese dioxide and concentrated sulphuric acid with boric acid, but the use of sodium 3-nitrobenzenesulfonate was much more efficient and convenient.

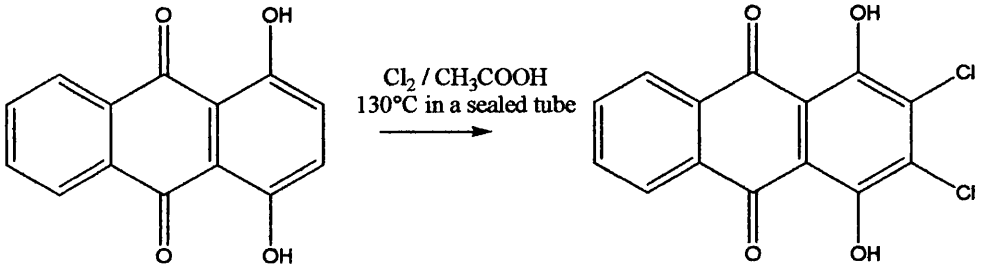
3.2 Chlorination of 1,4-dihydroxyanthracene-9,10-dione

The chlorination of 1,4-dihydroxyanthracene-9,10-dione **(111)** in acetic acid leads to the oxidation of 1,4-dihydroxyanthracene-9,10-dione to the tetra-quinone followed by the addition of one mole of chlorine[10] **(112)**.



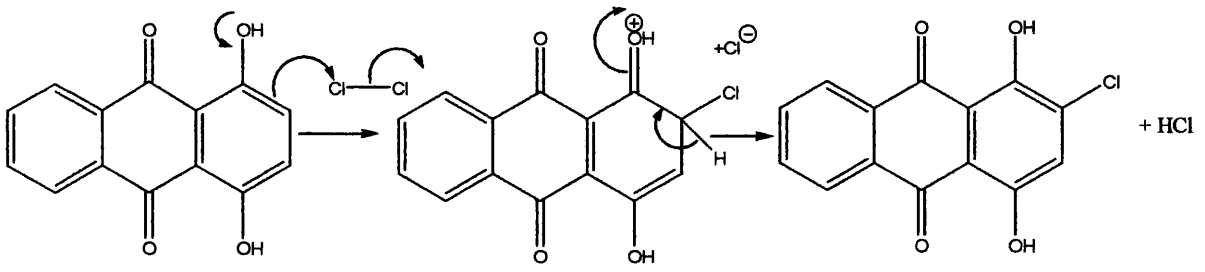
(111) **(112)**

If the same reaction is carried out in a sealed tube at 130°C, chlorination occurs at positions two and three of the anthracenedione nucleus, with the formation of 1,4-dihydroxy-2,3-dichloroanthracene-9,10-dione[10] **(113)**.



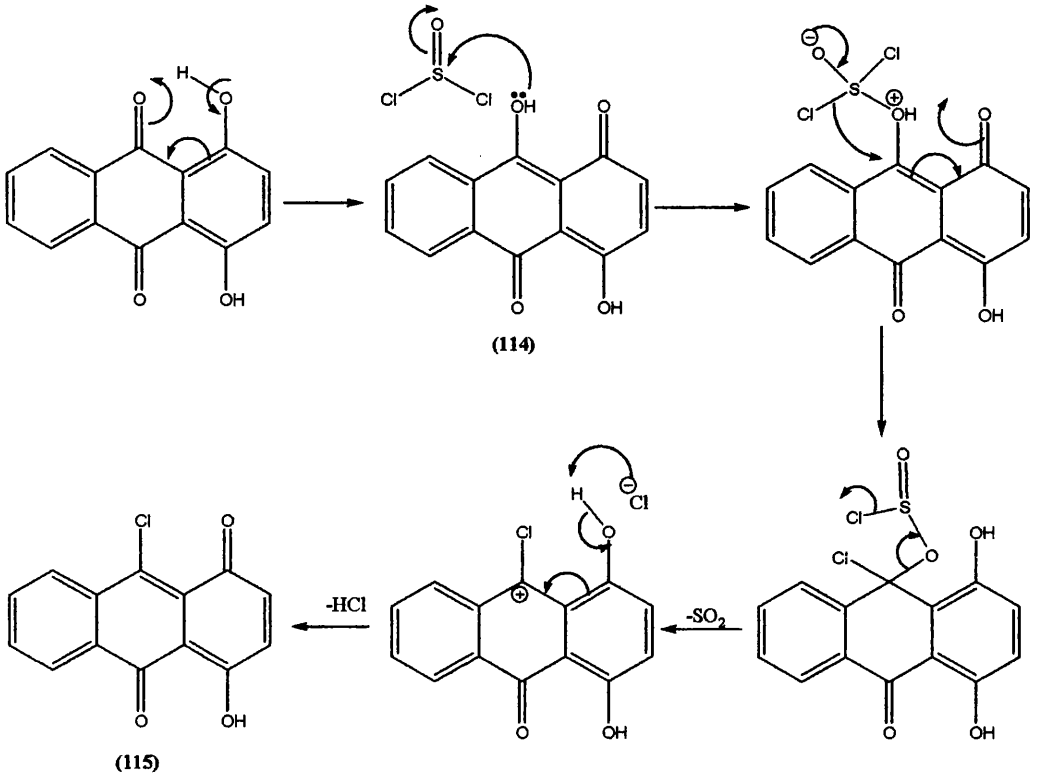
(113)

The chlorination at both the 2- and 3- positions of the anthracene-9,10-dione ring is expected due to the mesomeric effect of the hydroxyl groups which direct chlorination to these positions (Scheme 3-6).



Scheme 3-6. Chlorination mechanism for 1,4-dihydroxyanthracene-9,10-dione in acetic acid

If the reaction is carried out in DMF with the thionyl chloride under reflux for four hours 1,4-dihydroxy-2-chloroanthracene-9,10-dione is obtained. However, 1,4-dihydroxyanthracene-9,10-dione in boiling thionyl chloride results in the formation of 10-chloro-1-hydroxyanthracene-9-dione **(115)**. The literature suggests that 1,4-dihydroxyanthracene-9,10-dione exists in an ortho-quininiod **(114)** state, in which the two hydroxyl groups are in the one and ten positions[11] (Scheme 3-7).

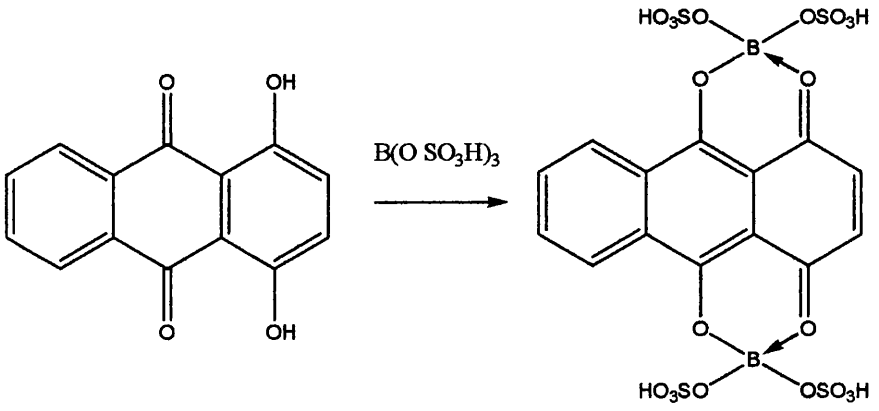


Scheme 3-7. (I) The action thionyl chloride on 1,4-dihydroxyanthracene-9,10-dione

The replacement of a hydroxyl group by a chlorine atom by means of thionyl chloride denotes an acidic proton and this is a well known method for the preparation of acyl chlorides from carboxylic acids. 10-Chloro-1-hydroxyanthracene-9-dione shows properties similar to that of an acyl chloride, such as the rapid reaction with amines.[11] The mechanism does not take into account why only one of the carbonyl groups of 1,4-dihydroxyanthracene-9,10-dione appears to take part in the reaction[11].

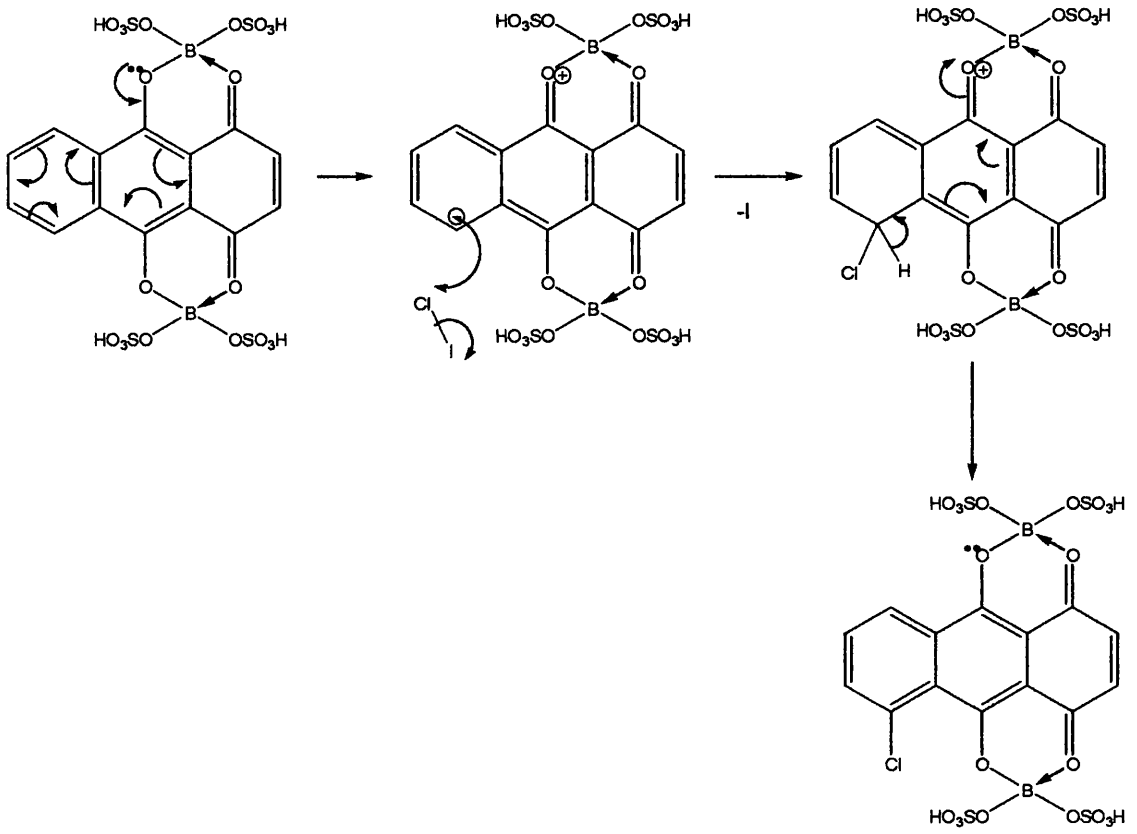
Synthesis of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione can be achieved by the chlorination of 1,4-dihydroxyanthracene-9,10-dione in 65% oleum and boric acid, with a catalyst of iodine. The reaction is thought to proceed via a boric acid intermediate **(116)**

which prevents oxidation and directs chlorination to the 5- and 8- positions. The chlorinating species in the reaction is iodine monochloride.



(116)

The boric acid intermediate **(116)** deactivates the right hand ring towards substitution but also activates the left hand ring towards substitution (Scheme 3-8).

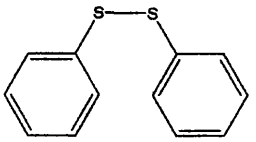


Scheme 3-8. Mechanism of chlorination of 1,4-dihydroxyanthracene-9,10-dione via boric ester intermediate

This mechanism does not account for the selectivity of chlorination at the 5- and 8-positions.

3.3 Reaction of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione with thiols

The attempted displacement of the chlorine atoms of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione with various thiols was unsuccessful. The reaction using sodium hydroxide and thiophenol in ethanol leads only to the formation of diphenyl disulphide (**117**).

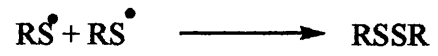


(117)

The formation of the disulphides is caused by molecular oxygen. This process occurs due the electron transfer from the thiol anion to molecular oxygen leading to the formation of a thiol radical and peroxide anion (Scheme 3-9)

Scheme 3-9. Reaction of thiolate ion with molecular oxygen

The thiol radical formed then goes on to react with another thiol radical which leads to the formation of the disulphide (Scheme 3-10).



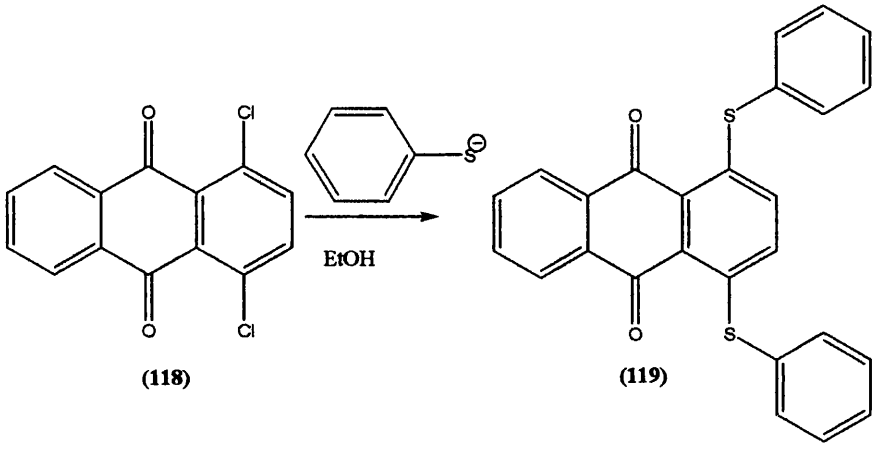
Scheme 3-10. Disulphide formation from thyinyl radicals

To avoid disulphide formation the reaction was carried out in a nitrogen atmosphere, although this did reduce the formation of the disulphide it did not prevent the disulphide formation. This can be attributed to 1,4-dihydroxy-5,8-dichloroanthracene-9,10-done acting as the electron accepting species, in place of molecular oxygen.

The formation of disulphides due to the presence of molecular oxygen has been studied extensively, as this type of reaction effects the stability of hydrocarbon fuels, since the oxidation of thiols found in these fuels leads to colour and sediment formation[12].

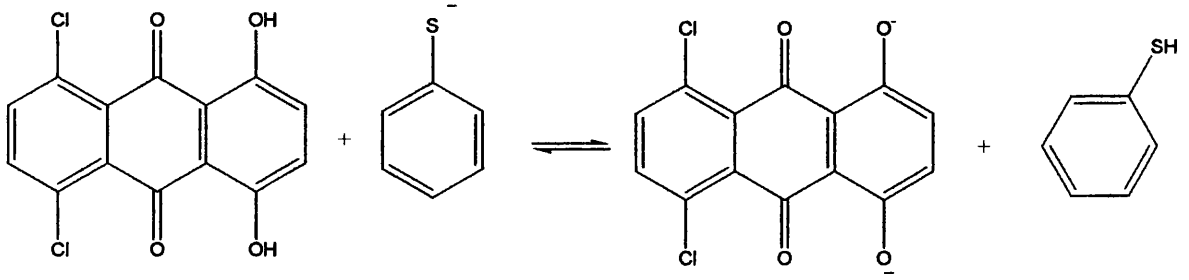
Reaction of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione in ethanol with other thiols such as 2-methyl-2-propanethiol and potassium thiocyanate was also unsuccessful and resulted in the recovery of the starting material. The base used in the reactions to generate the thiolate ion was also modified in type and concentration, for example potassium type bases such as potassium hydroxide and potassium carbonate have been shown to facilitate thiolation reactions that are difficult or don't occur when the analogous sodium type bases are employed[13] but this had no effect and only the starting material was recovered. Propanol was used to increase the reaction temperature: however this still didn't result in the formation of the desired product and only yielded the starting material. Polar aprotic solvents such as DMF or DMSO were also employed to make the thiolate ion more effective but again only the starting material was recovered from the reaction mixture.

Although this type of reaction is achievable with 1,4-dichloroanthracene-9,10-dione[14] **(118)** with thiophenol to yield 1,4-bis(phenylsulfanyl)anthracene-9,10-dione **(119)**, the presence of hydroxyl groups results in the molecule being less reactive towards nucleophilic substitution.



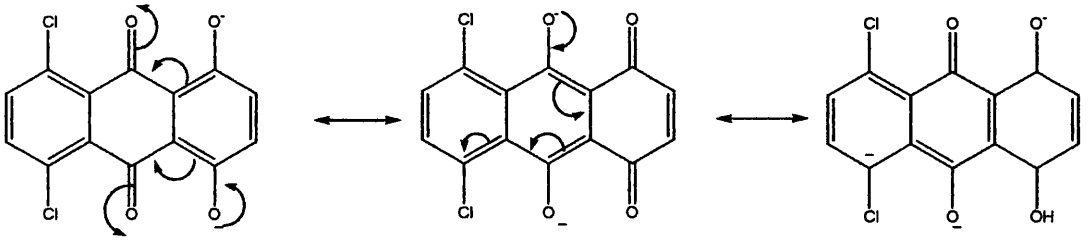
Scheme 3-11. Thiolation of 1,4-dichloroanthracene-9,10-dione

A similar observation was reported with reaction of 1,4-dichloro-8-hydroxy-5-methylanthracene-9,10-dione in which thiophenol does not displace the chlorine atoms[15]. It was also noted that the two chlorine atoms of 1,4-dichloro-8-hydroxy-5-methylanthracene-9,10-dione were not as easily displaced by phenoxy groups to yield 1,4-bis(phenoxy)-8-hydroxy-5-methylanthracene-9,10-dione in comparison to 1,4-dichloroanthracene-9,10-dione **(118)**. This decrease in reactivity can be attributed to delocalisation of the charge onto the quinone moiety, resulting in a lowered reactivity towards thiolation. The first step is abstraction of the acidic proton from the anthracene-9,10-dione (Scheme 3-12).



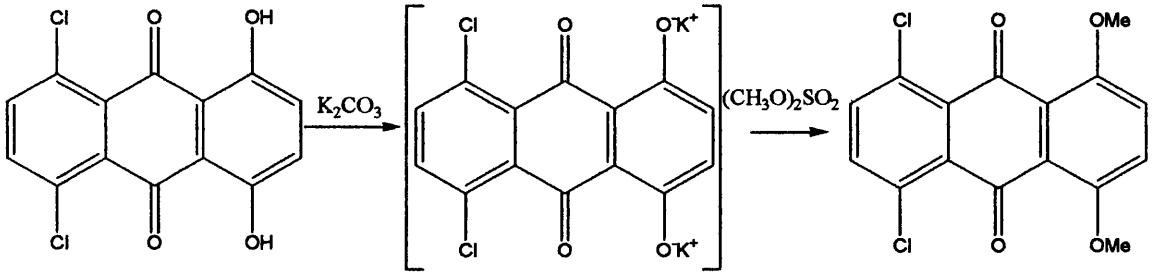
Scheme 3-12. Proton abstraction of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione

This then leads to resonance stabilized intermediate with the charge delocalised between the two oxygen atoms, which would reduce the reactivity of the chlorine atoms towards nucleophilic substitution.



Scheme 3-13. Delocalisation of charge onto the anthracene-9,10-dione moiety

In an attempt to prevent the delocalisation of the charge on to the quinone moiety, the hydroxyl groups were protected using dimethyl sulphate with potassium carbonate in acetone for 64 h[16] to give 1,4-dimethoxy-5,8-dichloroanthracene-9,10-dione **(120)**



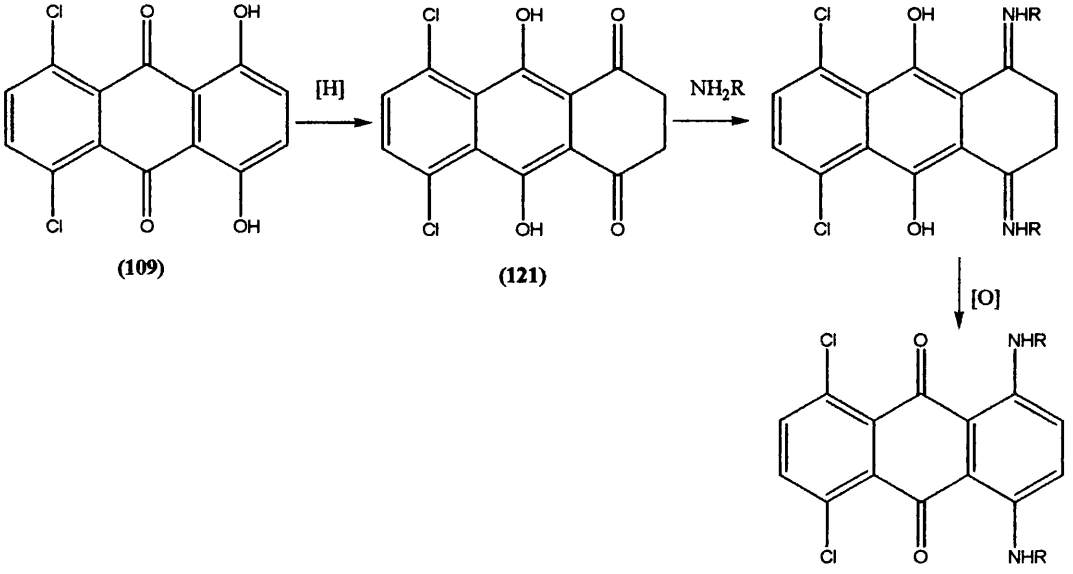
(120)

The thiolation of 1,4-dimethoxy-5,8-dichloroanthracene-9,10-dione was then attempted with thiophenol in ethanol, DMF and then DMSO but only the starting material was recovered. The base used to generate the thiolate was also modified (NaOH, KOH, Na_2CO_3 and K_2CO_3) but these reactions only yielded the starting material. The reactions were repeated using 2-methyl-2-propanethiol and potassium thiocyanate using ethanol,

DMF, and DMSO under reflux but all were unsuccessful and only yielded the starting materials.

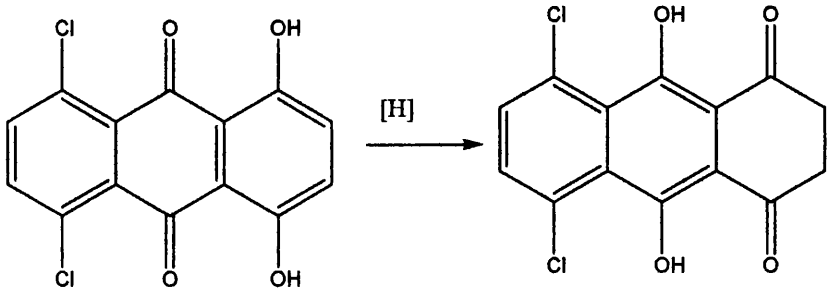
3.4 Reduction of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione

As the thiolation reactions were not achieved with 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione or 1,4-dimethoxyanthracene-9,10-dione with thiophenol, 2-methyl-2-propanethiol or potassium thiocyanate, another pathway was sought, due to the suspected deactivation of the molecule by the hydroxyl groups. It was decided next to eliminate this problem by reduction of the 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione **(109)** to 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione **(121)** followed by the subsequent amination



Scheme 3-14. Reduction and subsequent amination of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione

Reduction of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione can be achieved via tin in acetic acid[17] or sodium dithionite under alkali conditions[18], the latter being the more convenient method due to the faster reaction time.



(121)

Scheme 3-15. Reduction of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione

As with leuco 1,4-dihydroxyanthracene-9,10-dione, leuco 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione can exist in several tautomers, however the ^{1}H NMR (Figure 3-1) shows that it prefers to exist solely as the 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione tautomer (see section 3.1).

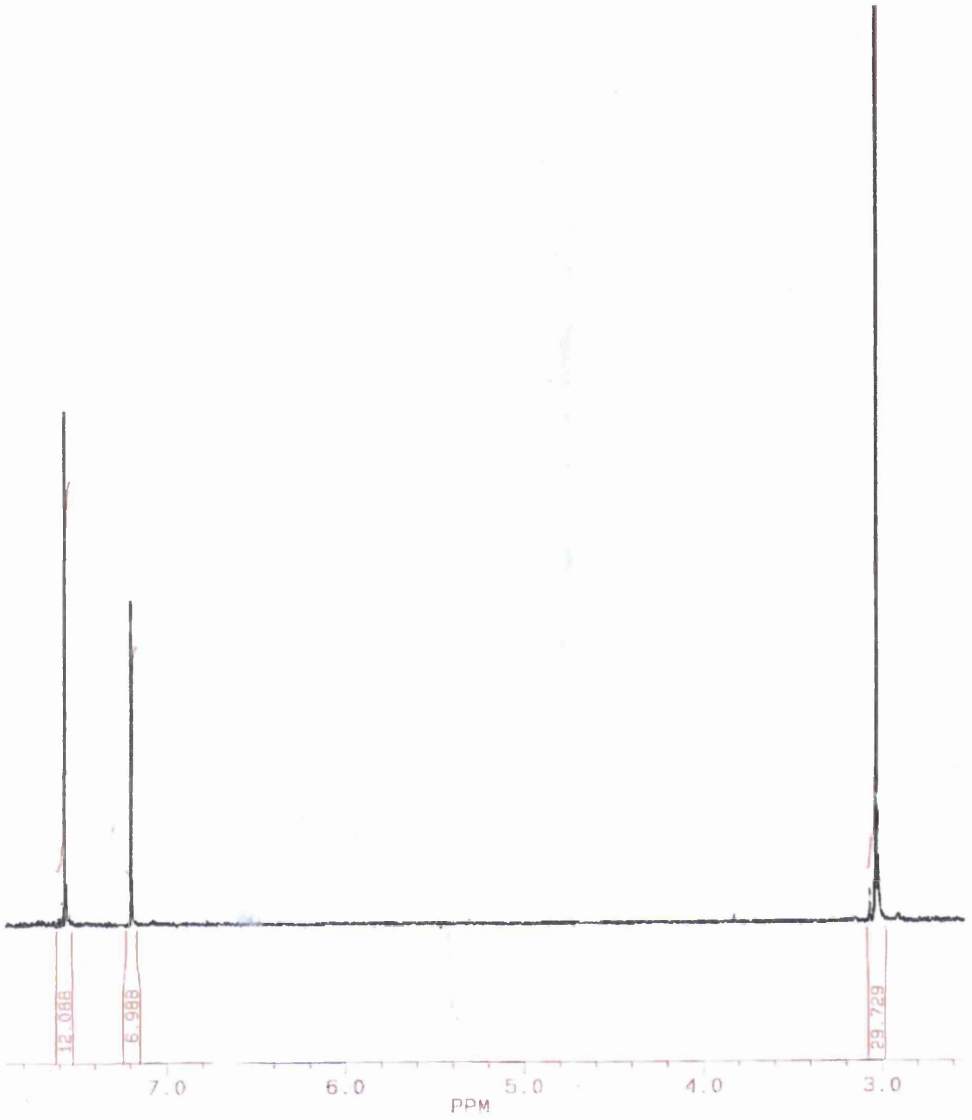
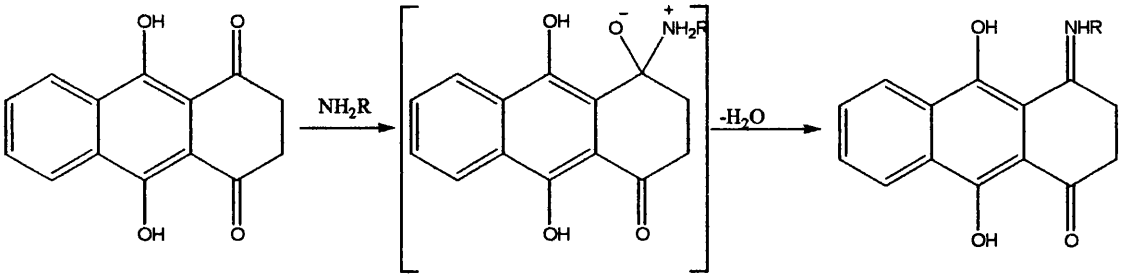


Figure 3-1. ^{1}H NHR for 2,3-dihydro-5,8-dichloro-9,10-dihydroxyanthracene-9,10-dione (in $CDCl_3$)

3.5 Amination of 9,10-dihydroxy-5,8-dichloro-2,3-dihydroanthracene-1,4-dione

The amination of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione can be achieved by reaction with the leuco or reduced form **(121)** followed by the subsequent air oxidation,

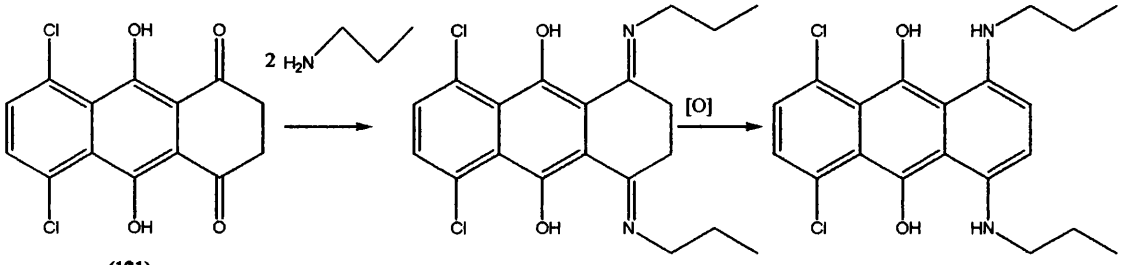
which is analogous to the amination of 1,4-dihydroxyanthracene-9,10-dione[19]. The reaction proceeds via an addition elimination mechanism, involving a polar intermediate[20] (Scheme 3-16).



Scheme 3-16. Amination of 2,3-dihydro-9,10-dihydroxyanthracene-1,4-dione

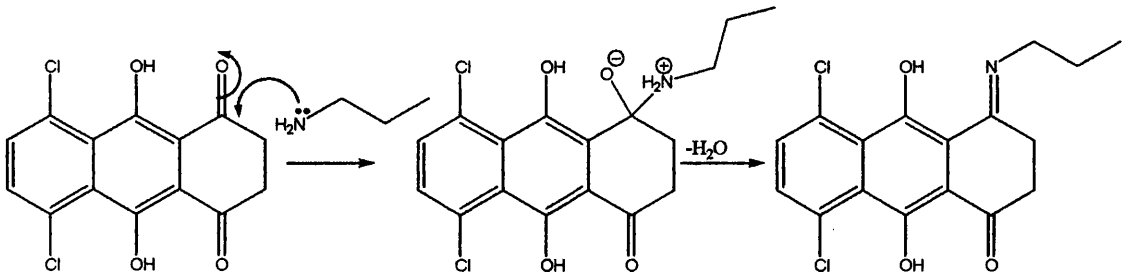
3.5.1 Amination of 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione with alkylamines

The amination of 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione **(121)** with an excess of propylamine to act as a solvent occurs readily to yield 1,4-bis(propylamino)-5,8-dichloroanthracene-9,10-dione **(123)** in 32% yield. The reaction is improved by employing a solvent such as ethanol which improves the workup and increases the yield to 48%.



(121) (122) (123)

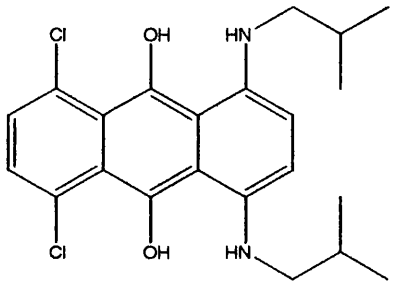
The reaction is an addition elimination mechanism followed by the air oxidation of the reduced intermediate **(123)**.



Scheme 3-17. Addition and elimination of propylamine to 2,3-dihydro-9,10-dihydroxyanthracene-9,10-dione

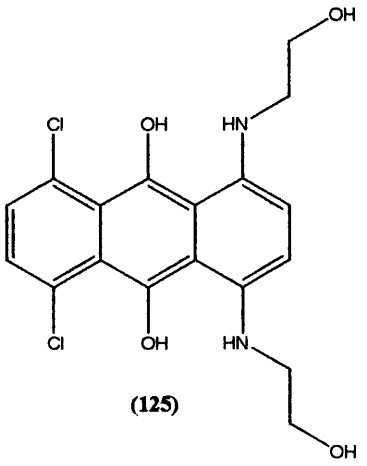
Attempts to isolate the reduced product by carrying out the reaction under a nitrogen atmosphere were unsuccessful. The oxidation of **(122)** takes place with relative ease and the reduced product is difficult to isolate, this is also the case for related 1,4-bis(alkylamino)anthracene-9,10-diones[21].

The reaction of 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione with isobutylamine also occurs readily to form 1,4-bis(isobutylamino)-5,8-dichloroanthracene-9,10-dione **(124)** in 52% yield.



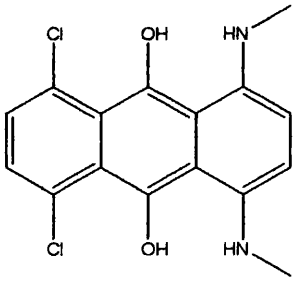
(124)

Similarly, the reaction of 2-aminoethanol with 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione in ethanol gives 1,4-bis(2-hydroxyethylamino)-5,8-dichloroanthracene-9,10-dione **(125)** in 64% yield.



(125)

Methylamine solution (20% in water) also reacts readily with 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione to yield 1,4-bis(methylamino)-5,8-dichloroanthracene-9,10-dione **(126)** in 43% yield.

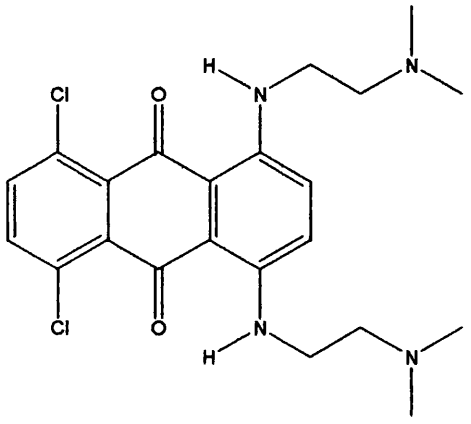


(126)

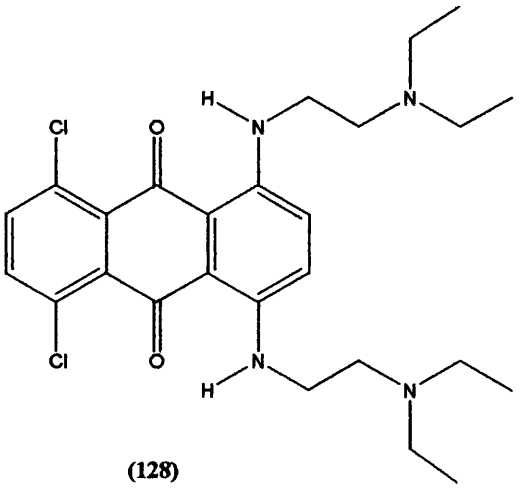
The reaction of 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione with amines containing a secondary nitrogen atom especially 2-[(2-aminoethyl)amino]ethanol leads to problems at the purification stage. The reaction mixtures yield tar-like solids that were difficult to purify, especially since amino-anthracene-9,10-diones are known to be difficult to purify by normal methods[22,23].

The amination of 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione with N,N-dimethylethylenediamine and N,N-diethylethylenediamine also occurs to give 1,4-bis{[2-(dimethylamino)ethyl]amino}-5,8-dichloroanthracene-9,10-dione (**127**) and 1,4-

bis{[2-(diethylamino)ethyl]amino}-5,8-dichloroanthracene-9,10-dione (**128**) in 29% and 22% yield respectively.



(127)



(128)

The ^{1}H NMR data for all the 1,4-bis(alkylamino)-5,8-dichloroathracene-9,10-diones shows that the peak attributed to the *NH* proton is split into a triplet.

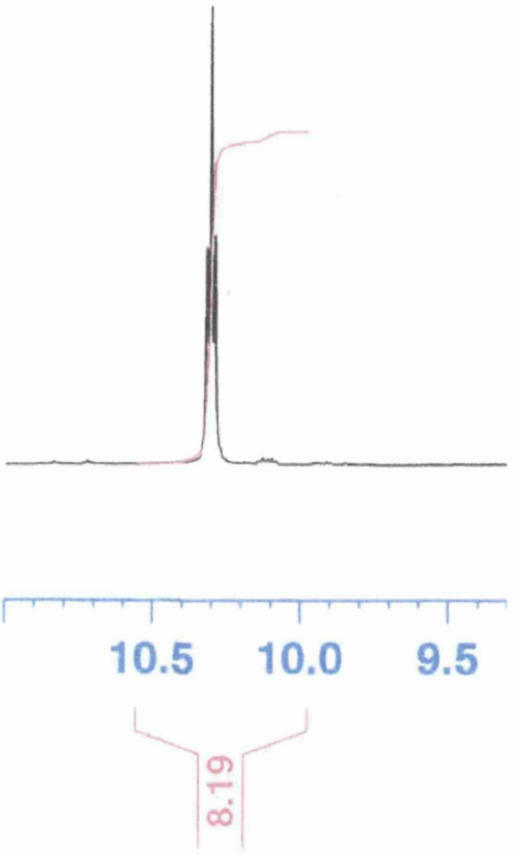
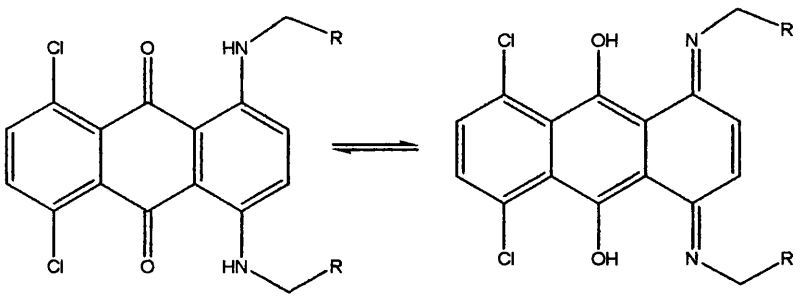


Figure 3-2. Splitting pattern for NH proton

This is of significance as it confirms the tautomeric preference of 1,4-bis(alkylamino)-5,8-dichloroathracene-9,10-diones is structure (I) in Figure 3-2 (see section 3.1).

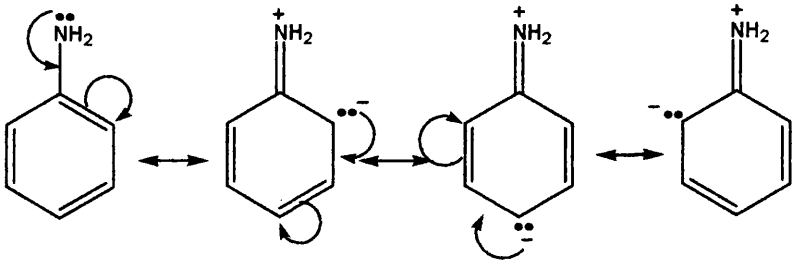


(I) (II)

Scheme 3-18. Potential tautomers of 1,4-bis(alkylamino)-5,8-dichloroathracene-9,10-diones.

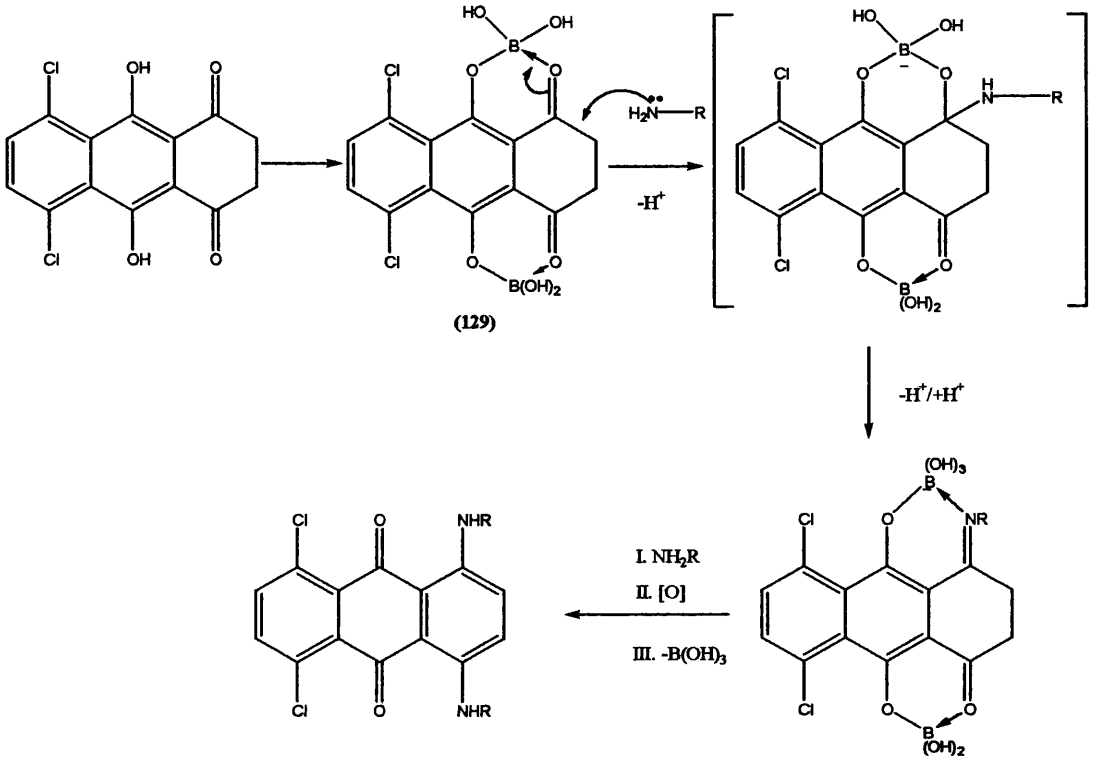
3.5.2 Amination of 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione with aniline

The amination of 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione with aniline was unsuccessful under varying conditions but the reaction can be achieved if boric acid is used as a catalyst. This lack of reactivity towards 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione as compared to the other alkylamines used can be attributed to the fact that aniline is a weaker nucleophile due to delocalisation of the electron lone pair of the nitrogen atom into the aromatic ring (Scheme 3-19).

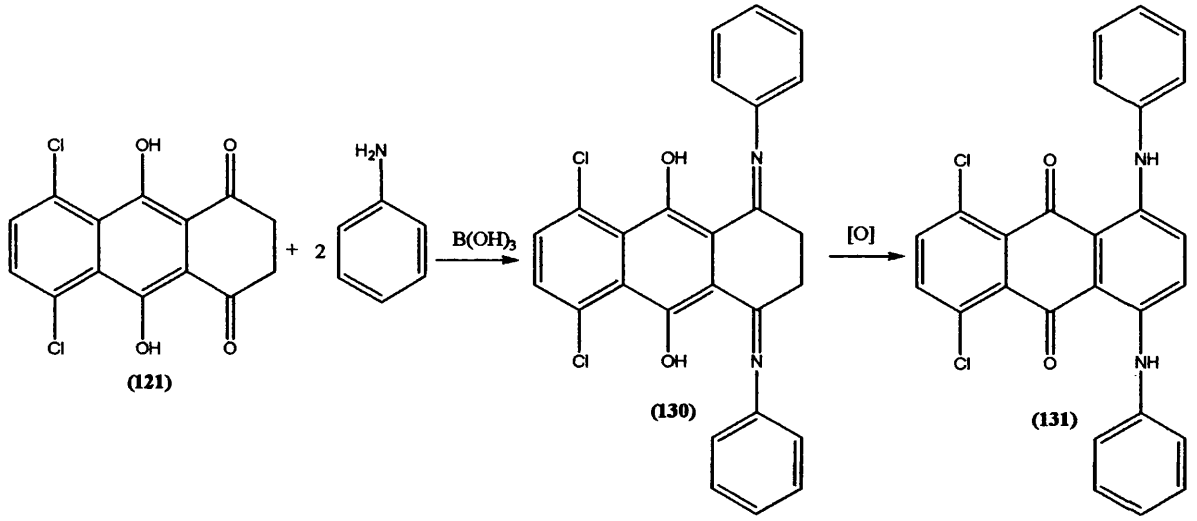


Scheme 3-19. Delocalisation of the electron pair in aniline

Thus the addition of boric acid as a catalyst is required to facilitate the reaction with the formation of a boric ester intermediate **(129).**

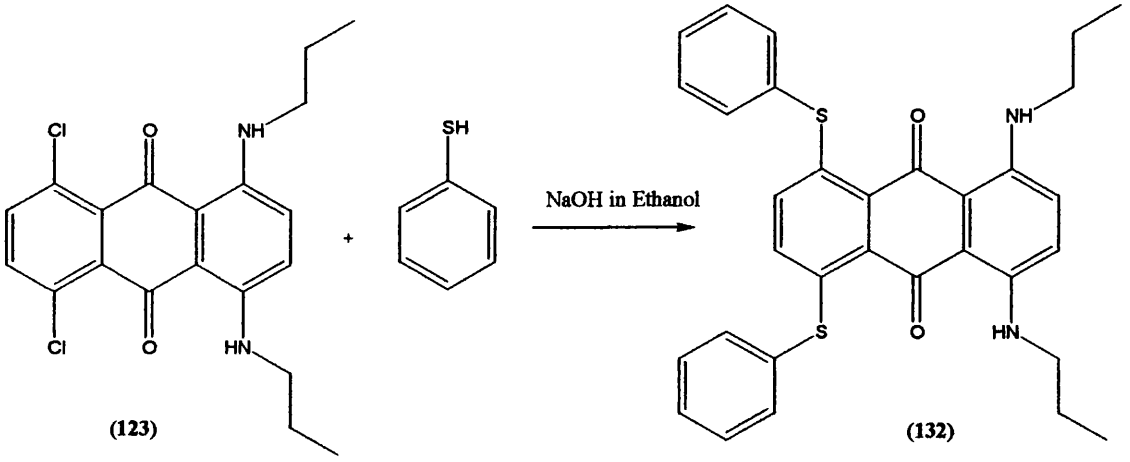


Thus the synthesis of 1,4-bis(phenylamino)-5,8-dichloroanthracene-9,10-dione **(131)** was achieved from the reaction of 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione **(119)** with aniline in the presence of boric acid.



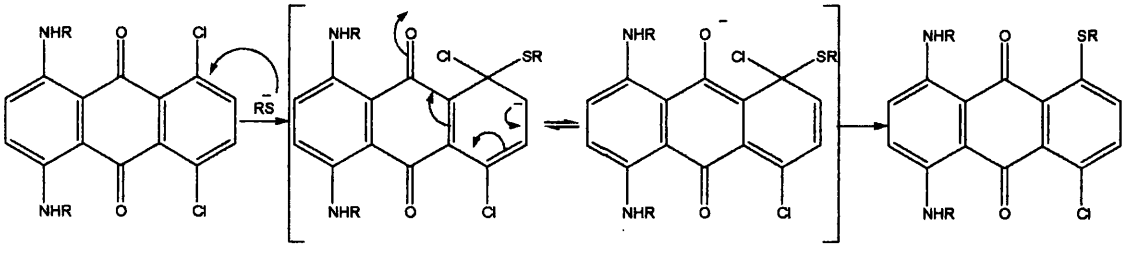
3.6 Thiolation of 1,4-bis(alkylamino)-5,8-dichloroathracene-9,10-diones

Despite the deactivating effects of the alkylamino groups, the reaction of 1,4-bis(propylamino)-5,8-dichloroanthracene-9,10-dione **(123)** with thiophenol in ethanol and an appropriate base gives 1,4-bis(propylamino)-5,8-bis(phenylsulfanyl)anthracene-9,10-dione **(132)** in 41% yield. The yield of the reaction is improved to 66% by employing DMF as the solvent.

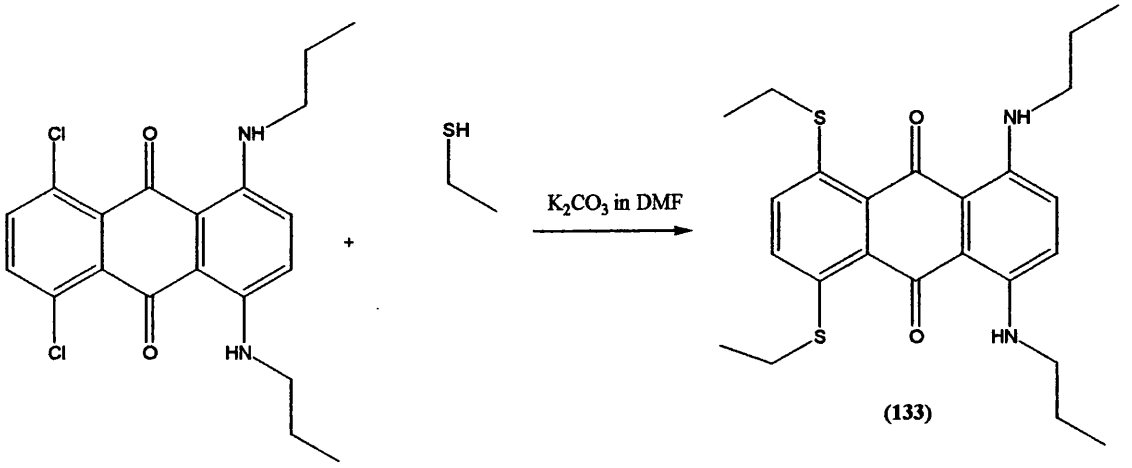


(123) (132)

The reaction is a nucleophilic addition elimination reaction (S_NAr Mechanism) involving the formation of a delocalised intermediate.

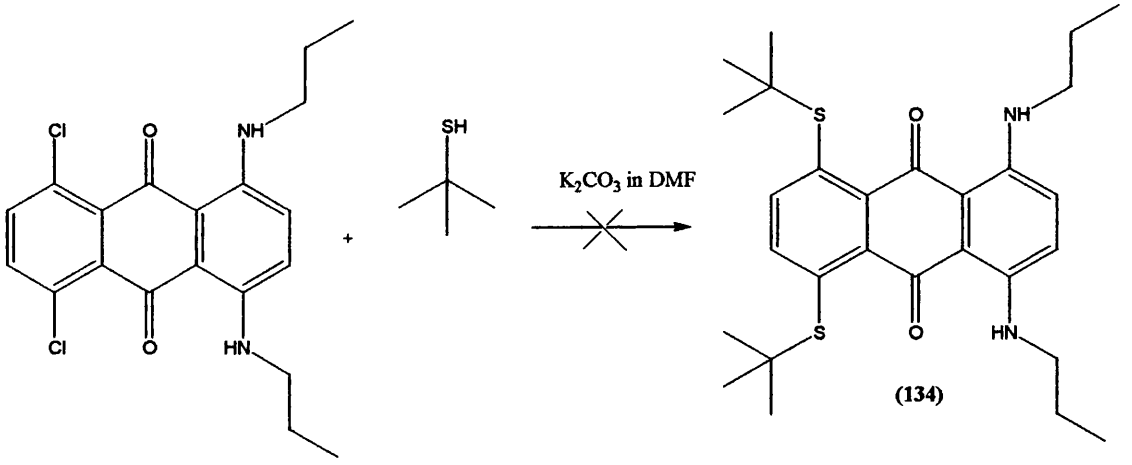


However, the reaction of 2-ethanethiol under similar conditions yields the 1,4-bis(propylamino)-5,8-bis(ethylsulfanyl)anthracene-9,10-dione **(133)** in 62 % yield.



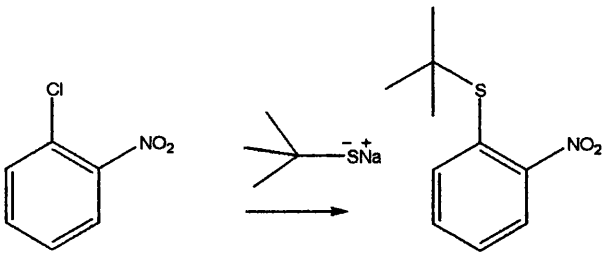
(133)

The reaction of 2-methyl-2-propanethiol with 1,4-bis(propylamino)-5,8-dichloroanthracene-9,10-dione in ethanol doesn't yield the expected 1,4-bis(aminopropyl)-5,8-bis(2-methyl-2-propanethiol)anthracene-9,10-dione (**134**).



(134)

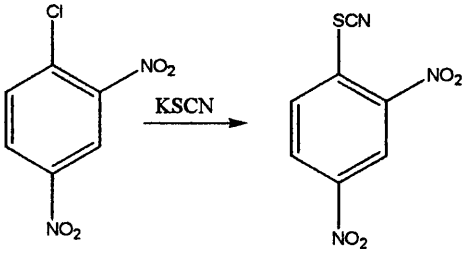
Although 2-methyl-2-propanethiol will react with activated benzenes such as 1-chloro-2-nitrobenzene (**135**) in 2-propanol as the sodium salt at 50°C to yield 1-(tert-butylsulfanyl)-2-nitrobenzene (**136**)[24],



(135) (136)

the use of DMF or DMSO for the 1,4-bis(propylamino)-5,8-dichloroanthracene-9,10-dione reaction still doesn't facilitate the reaction, nor does the modification of the base used. The lack of reaction in this case can be attributed to the steric effects of the nucleophile. The sulphur atom of 2-methyl-2-propanethiol is sterically hindered by the three surrounding methyl groups making it a less effective nucleophile.

The reaction of potassium thiocyanate with 1,4-bis(propylamino)-5,8-dichloroanthracene-9,10-dione in various solvents was also fruitless, even though potassium thiocyanate does undergo reaction with activated chlorobenzenes[25] such as 1-chloro-2,4-dinitrobenzene (**137**) to yield 2,4-dinitrophenyl thiocyanate (**138**).



(137) (138)

The lack of reaction may be attributable to the poor nucleophilic character of the attacking thiocyanate, due to the delocalisation of charge across the molecule.

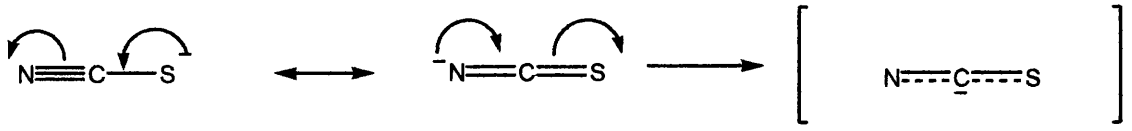
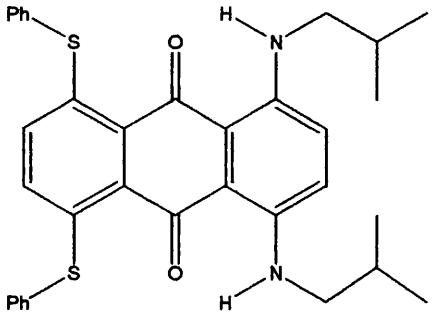
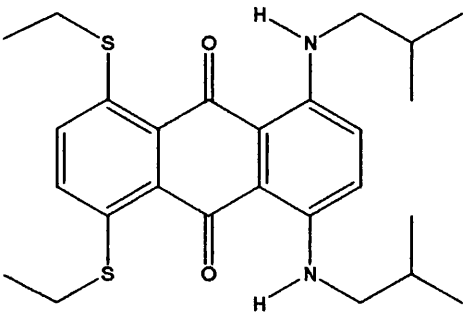


Figure 3-3 Charge delocalisation in thiocyanate nucleophile

1,4-Bis(isobutylamino)-5,8-dichloroanthracene-9,10-dione also under goes thiolation with thiophenol and 2-ethanethiol to yield 1,4-bis(isobutylamino)-5,8-bis(phenylsulfanyl)-9,10-dione (**139**) and 1,4-bis(isobutylamino)-5,8-bis(ethylsulfanyl)-9,10-dione (**140**) in 37% and 41% yield respectively. However, there is no reaction between 1,4-bis(isobutylamino)-5,8-dichloroanthracene-9,10-dione with either 2-methyl-2-propanethiol or potassium thiocyanate.

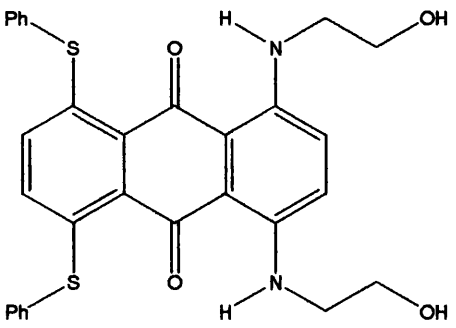


(139)

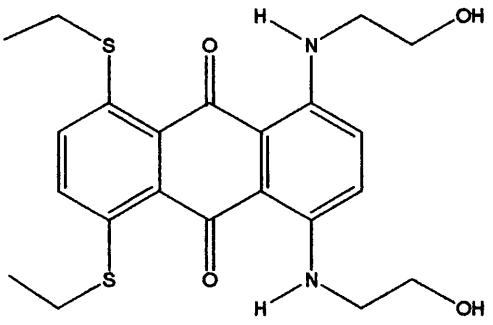


(140)

Similarly, thiolation of 1,4-bis[2-(hydroxyethyl)amino]-5,8-dichloroanthracene-9,10-dione with thiophenol and 2-ethanethiol in DMF gives 1,4-bis[2-(hydroxyethyl)amino]-5,8-bis(phenylsulfanyl)anthracene-9,10-dione (**141**) and 1,4-bis[2-(hydroxyethyl)amino]-5,8-bis(ethylsulfanyl)anthracene-9,10-dione (**142**) in 67% and 51% yield respectively. Again no reaction is observed with 2-methyl-2-propanethiol and potassium thiocyanate.

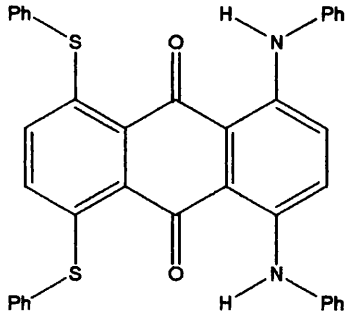


(141)

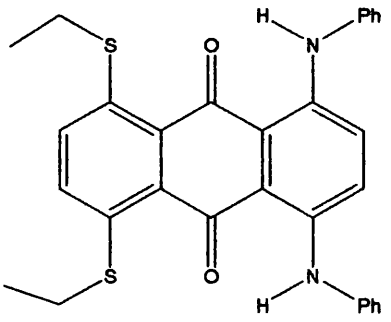


(142)

1,4-Bis(phenylamino)-5,8-bis(phenylsulfanyl)anthracene-9,10-dione (**143**)[26] and 1,4-bis(phenylamino)-5,8-bis(ethylsulfanyl)anthracene-9,10-dione (**144**) were synthesised in yields of 60% and 52% respectively, from 1,4-bis(phenylamino)-5,8-dichloroanthracene-9,10-dione and the appropriate thiols.



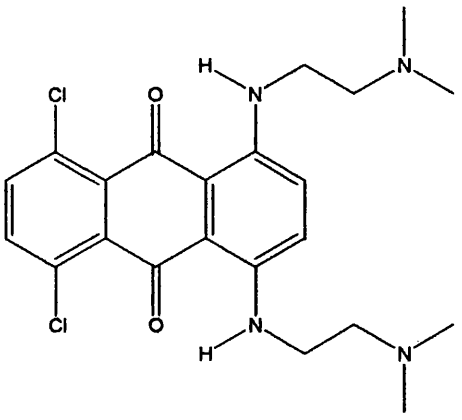
(143)



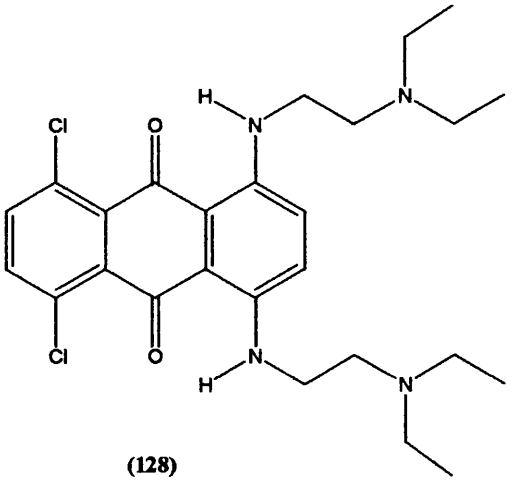
(144)

Again no reaction occurred between either 2-methyl-2-propanethiol or potassium thiocyanate with 1,4-bis(phenylamino)-5,8-dichloroanthracene-9,10-dione.

The thiolation of 1,4-bis{[2-(dimethylamino)ethyl]amino}-5,8-dichloroanthracene-9,10-dione (**127**) and 1,4-bis{[2-(diethylamino)ethyl]amino}-5,8-dichloroanthracene-9,10-dione (**128**) with thiophenol and ethanethiol was unsuccessful in both ethanol and DMF, and only led to recovery of the starting materials.

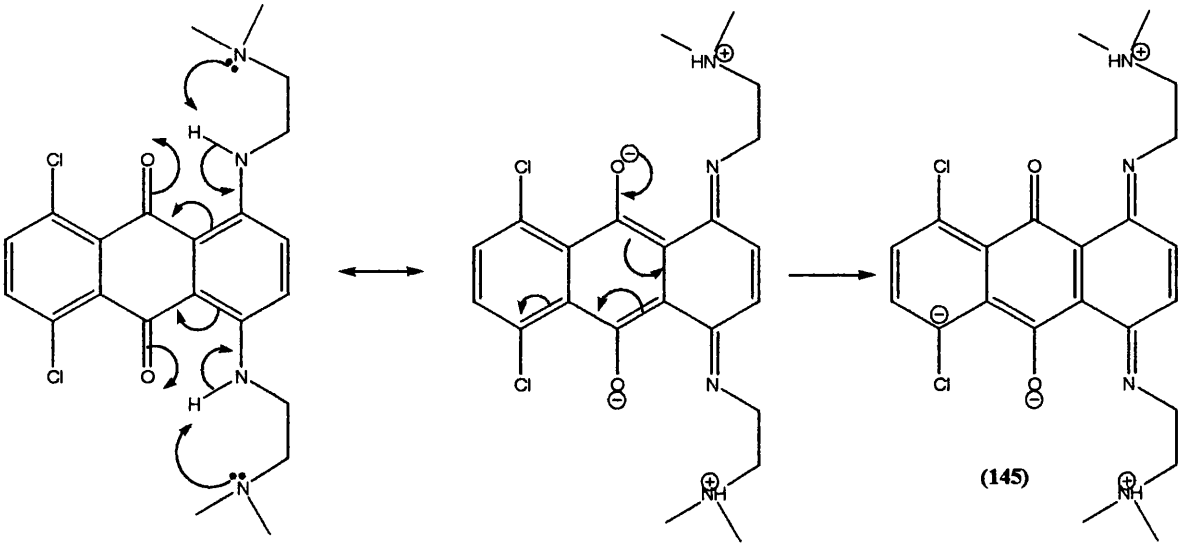


(127)



(128)

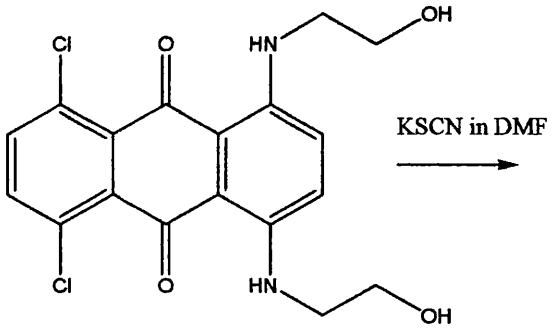
This can only be attributed to the presence of the nitrogen atom in the side chain. This may be causing solubility problems or there may be electronic factors at work. For example abstraction of the proton from the amino group, leads to delocalisation of negative charge on to the ring carbon next to the chlorine atoms, making the molecule less reactive towards attacking nucleophiles (**145**).



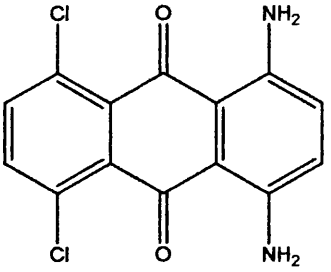
(145)

3.7 Autoxidation of (alkylamino)anthracene-9,10-diones

The attempted thiolation of 1,4-bis(alkylamino)-5,8-anthracene-9,10-diones in DMF for prolonged periods lead to some interesting results. Firstly, the 1,4-bis(aminoethanol)-5,8-dichloroanthracene-9,10-dione (**125**) heated in DMF with potassium thiocyanate did not produce the expected 1,4- diisothiocynato-5,8- bis[2-(hydroxyethyl)amino]anthracene-9,10-dione even after 36 hours, but dealkylation occurs to give 1,4-diamino-5,8-dichloroanthracene-9,10-dione (**146**), which was recovered from the reaction mixture.



(125)

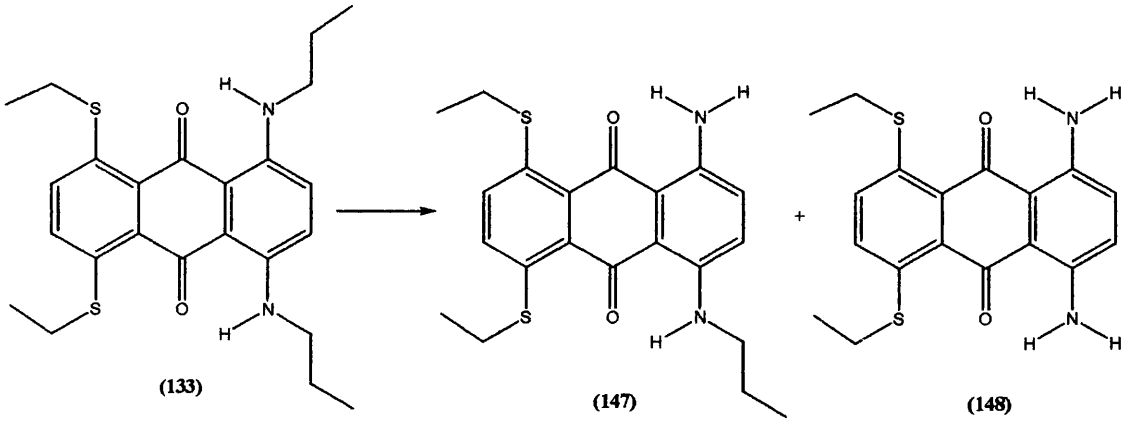


(146)

1,4-Bis[2-(hydroxyethyl)amino]-5,8-dichloroanthracene-9,10-dione (**125**) was heated in DMF to asses the importance of the thiol in the decomposition reaction, this had no effect indicating that the thiol plays no part in the decomposition of 1,4-bis[2-(hydroxyethyl)amino]-5,8-dichloroanthracene-9,10-dione (**125**).

The reaction of 1,4-bis(propylamino)-5,8-dichloroanthracene-9,10-dione (**123**) with 2-ethanethiol and potassium hydroxide in DMF yields 1,4-bis(propylamino)-5,8-bis(ethylsulfanyl)anthracene-9,10-dione (**133**), but if the reaction is allowed to continue two other products are produced, 1-(propylamino)-4-amino-5,8-

bis(ethylsulfanyl)anthracene-9,10-dione (**147**) and 1,4-diamino-5,8-bis(ethylsulfanyl)-9,10-dione (**148**).



(133) (147) (148)

A similar type of reaction occurs when amino containing dyes are faded by light[27]. The reaction is attributed to singlet oxygen and is widely acknowledged as a fading mechanism for many amino containing dyes[27]. One possible mechanism for the reaction is shown in (Figure 3-4).

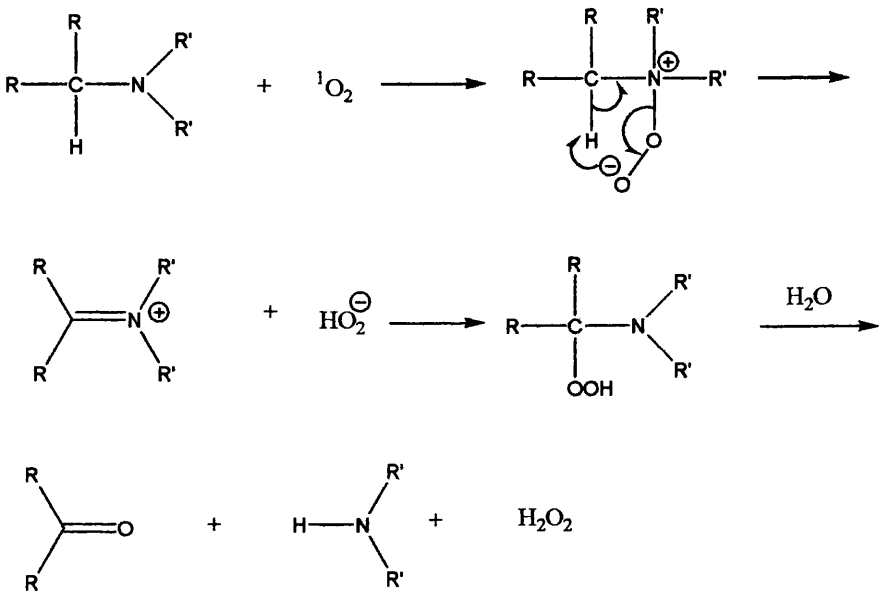
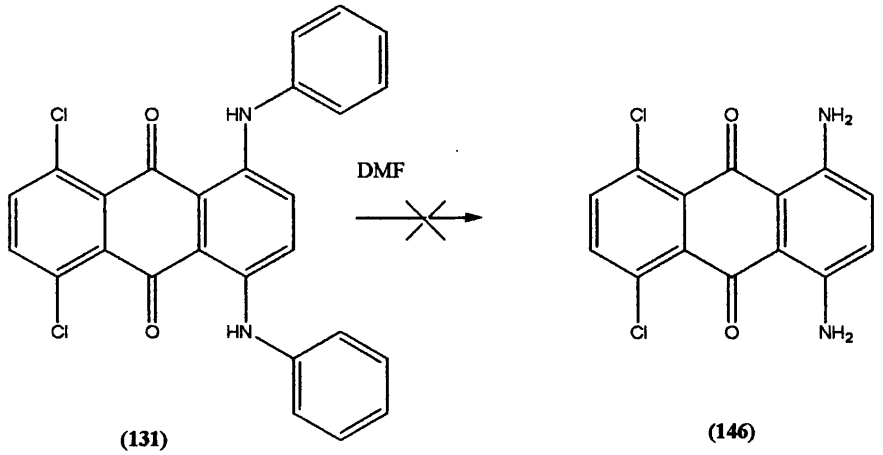


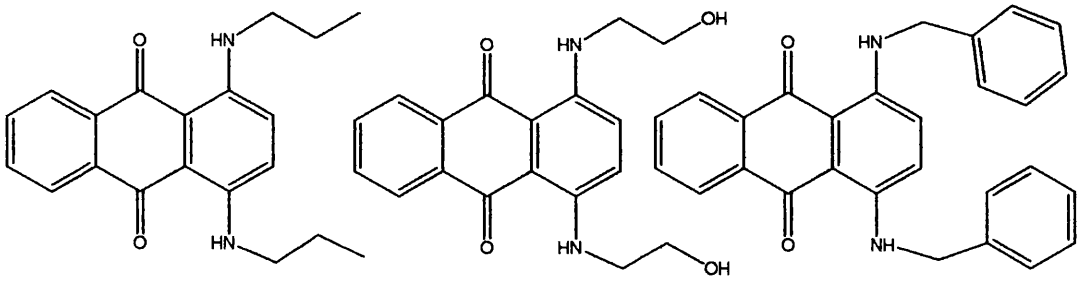
Figure 3-4. Autoxidation of amino compounds by singlet oxygen

Singlet oxygen ($^1O_2$) is a higher energy state of molecular oxygen and is one of the most active intermediates in biological reactions. It was first observed in 1924 and defined as a more reactive species of oxygen[28]. Singlet oxygen is generated when two unpaired electrons of molecular oxygen in its ground state rearrange and become paired in a single orbit. This excited state is 23 kcal mol^{-1} higher in energy than ground state. It can be seen from the reaction mechanism, that the initial proton abstraction occurs α to the nitrogen atom and therefore a hydrogen atom must be present for oxidation to take place. Taking this into consideration 1,4-bis(phenylamino)-5,8-dichloroanthracene-9,10-dione **(131)** was heated in DMF for prolonged periods of time and did not yield 1,4-diamino-5,8-dichloroanthracene-9,10-dione.



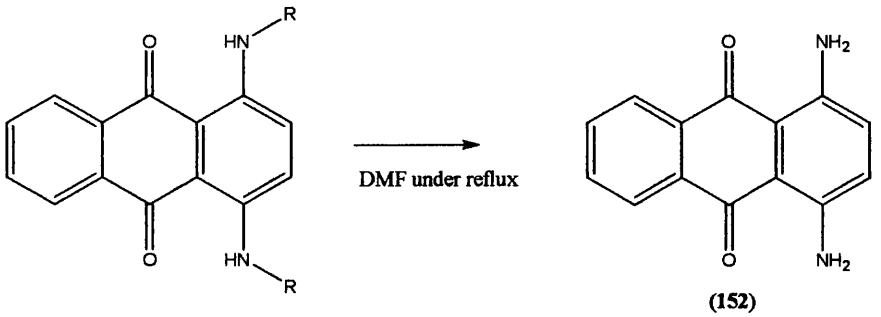
(131) (146)

In an attempt to asses the importance of the chlorine atom present at the five and eight positions 1,4-bis(propylamino)anthracene-9,10-dione (**149**), 1,4-bis[2-(hydroxyethyl)amino]anthracene-9,10-dione (**150**), 1,4-bis(benzylamino)anthracene-9,10-dione (**151**) were synthesised from 2,3-dihydro-9,10-dihydroxyanthracene-9,10-dione.



(149) (150) (151)

1,4-Bis(propylamino)anthracene-9,10-dione (**149**), 1,4-bis[2-(hydroxyethyl)amino]anthracene-9,10-dione (**150**), and 1,4-bis(benzylamino)anthracene-9,10-dione (**151**) were refluxed in DMF and all resulted in dealkylation to give 1,4-diaminoanthracene-9,10-dione (**152**).



(152)

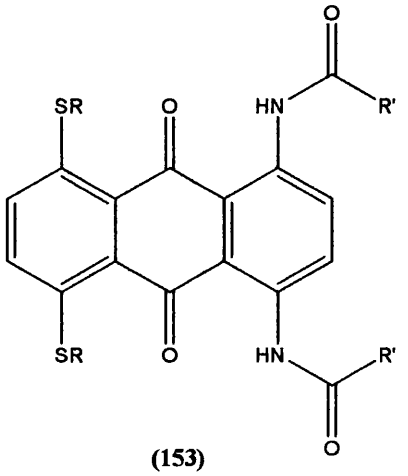
When the reactions were repeated under a nitrogen atmosphere, this prevented the formation of the degradation product, suggesting that oxygen was essential for the degradation to occur. Conversely when hydrogen peroxide was added to the reaction mixtures the degradation was accelerated. The addition of hydrogen peroxide is a good indicator that the reaction is of a free radical nature.

To asses the role of light in the decomposition reaction the afore mentioned reactions were repeated in the absence of daylight, however this had no effect.

As light plays no part in the reaction the generation of a reactive species must occur through some other pathway. The most likely explanation is that the anthracene-9,10-dione moiety is undergoing a one electron reduction to form the anthracene-9,10-dione radical. This can then undergo an electron transfer reaction with molecular oxygen to form the superoxide anion[29]. The superoxide radical could then undergo a nucleophilic addition elimination reaction leading to the degradation of the amino side chain.

3.8 Synthesis of amido analogous

The synthesis of amido analogous with the general formula **(153)** was also attempted.

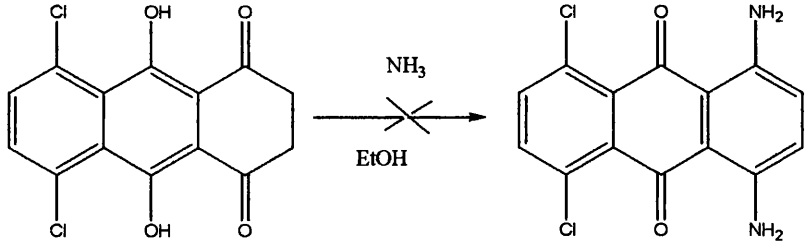


(153)

Amido analogous such as **(153)** should be obtainable from the acylation of 1,4-diamino-5,8-dichloroanthracene-9,10-dione.

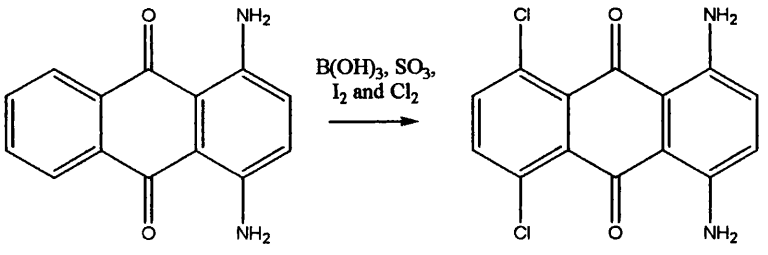
Two methods were selected for the synthesis of 1,4-diamino-5,8-dichloroanthracene-9,10-dione (a) amination of 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-9,10-dione using ammonia and (b) chlorination of 1,4-diaminoanthracene-9,10-dione **(148)**.

The amination was attempted by bubbling ammonia gas through an ethanol solution of 2,3-dihydro-9,10-dihydroxyanthracene-9,10-dione heated to 50°C, but no reaction occurred.



(121) **(146)**

In an alternative strategy 1,4-diaminoanthracene-9,10-dione **(152)** was chlorinated using boric acid, 65% oleum, iodine and a chlorinating agent as described previously for 1,4-dihydroxyanthracene-9,10-dione (see section 3.10).



(152) **(146)**

Analysis of the product showed that a mixture of chlorination products was present, which was difficult to separate effectively. Acylation of the mixed chlorination product was attempted with benzoyl chloride but led to an intractable tar.

3.9 Experimental

3.9.1 Oxidation of 2,3-dihydro-9,10-dihydroxyanthracene-1,4-dione

3.9.2 Using nitrobenzene

2,3-Dihydro-9,10-dihydroxyanthracene-1,4-dione (0.50g, 2.1 mmol) was refluxed for 19 h in nitrobenzene (10 ml). The nitrobenzene was removed by steam distillation, the residue were collected by filtration, washed with water and air-dried. The solid was recrystallised from glacial acetic acid (8 ml) to give 1,4-dihydroxy-9,10-anthracene-9,10-dione (0.30g, 59%), mp 190°C, (lit 200-2°C [30]), δ_H ($CDCl_3$) 7.2(2H,s), 7.8(2H,m), 8.2(2H,m), 12.8(2H,*OH*,s)

3.9.3 Using sodium 3-nitrobenzenesulphonate

2,3-Dihydro-9,10-dihydroxyanthracene-1,4-dione (1.00g, 4.1 mmol) and 3-nitrobenzenesulphonate (10.0g, 49 mmol) were added to a solution of sodium hydroxide (1.00g, 25 mmol) in water (50 ml). The mixture was heated under reflux for 2 h and the solution was allowed to cool. It was acidified with conc. sulphuric acid (10 ml), the precipitate was filtered off and dried. The solid was then recrystallised from glacial acetic acid (15 ml) to give 1,4-dihydroxy-9,10-anthracene-9,10-dione (0.6g, 61%), mp 192°C, (lit 200-2°C[30]), δ_H ($CDCl_3$) 7.2(2H,s), 7.8(2H,m), 8.2(2H,m), 12.8(2H,*OH*,s)

3.9.4 Using concentrated sulphuric acid

2,3-Dihydro-9,10-dihydroxyanthracene-1,4-dione (1.00g, 4.1 mmol), boric acid (0.30g, 4.8 mmol) and concentrated sulphuric acid (5 ml) was heated to 50°C for 24 h. The

solution was cooled, diluted with water (50 ml) and the precipitate was filtered off and recrystallised form glacial acetic acid (15 ml) to give 1,4-dihydroxy-9,10-anthracene-9,10-dione (0.40g, 41%), mp 187°C, (lit 200-2°C[30]), δ_H ($CDCl_3$) 7.2(2H,s), 7.8(2H,m), 8.2(2H,m), 12.8(2H,*OH*,s)

3.9.5 Using activated manganese dioxide

Activated manganese dioxide (0.50g, 5.9 mmol) was added to a solution of 2,3-dihydro-9,10-dihydroxyanthracene-1,4-dione (0.35g, 1.4 mmol) in chloroform (25 ml). The reaction mixture was stirred at room temperature for 48h, the manganese dioxide was removed by filtration and the filtrate was concentrated by rotary evaporation to yield 1,4-dihydroxy-9,10-anthracene-9,10-dione(0.28g, 81%) %), mp 189°C, (lit 200-2°C[30]), δ_H ($CDCl_3$) 7.2(2H,s), 7.8(2H,m), 8.2(2H,m), 12.8(2H,*OH*,s)

3.10 Chlorination of 1,4-dihydroxyanthracene-9,10-dione

3.10.1 Using thionyl chloride

1,4-Dihydroxyanthracene-9,10-dione (5.0g, 20.1 mmol) was refluxed in thionyl chloride (40.0g, 30 mmol) for 2 h and allowed to cool, a mixture of boric acid (2.6g, 4.2 mmol), 65% oleum (40g)and iodine (1.00g, 3.9 mmol) was added. The mixture was heated to 70°C for 24 h and then poured onto ice (1000g), the precipitate was collected by filtration, washed with water, and recrystallised from DMF (150 ml) to give 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione (1.8g, 28%), mp 272-3 °C, (lit 283-4°C[31]), δ_H ($CDCl_3$) 7.3(2H,s), 7.7(2H,s), 12.6(2H,*OH*,s), *m/z* (EI) molecular ion 308.

3.10.2 Using chlorine gas

1,4-Dihydroxyanthracene-9,10-dione (5.0g, 20.1 mmol) was added to a mixture of boric acid (2.6g, 4.2 mmol), 65% oleum (80g) and iodine (1.00g, 3.9 mmol). The mixture was heated to 70°C for 24 h, while chlorine gas was bubbled through. The mixture was allowed to cool and poured onto ice (1000g), the resulting precipitate was collected by filtration, washed with water and recrystallised from DMF (150 ml) to give 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione (2g, 31%), mp 270-2 °C, (lit 283-4°C[31]), δ_H ($CDCl_3$) 7.3(2H,s), 7.7(2H,s), 12.6(2H,*OH*,s), *m/z* (EI) 308.

Chlorine gas was generated by the drop wise addition of concentrated hydrochloric acid (35 ml) to potassium permanganate (5.0g) from a pressure equalising funnel. The chlorine gas evolved was passed through a solution of water and then concentrated sulphuric acid.

3.10.3 Using chlorosulphonic acid

1,4-Dihydroxyanthracene-9,10-dione (5.0g, 20.1 mmol) was added to a mixture of boric acid (2.6g, 4.2 mmol), 65% oleum (80.0g), chlorosulphonic acid (80 ml, 0.3 mol) and iodine (1.00g, 3.9 mmol). The mixture was heated to 70°C for 24 h. The mixture was allowed to cool, poured onto ice (1000g), the precipitate was collected by filtration, washed with water and dried. The solid was recrystallised from DMF (150 ml) to yield 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione (3.00g, 47%), mp 276-8 °C, (lit 283-4°C[31]), δ_H ($CDCl_3$) 7.3(2H,s), 7.7(2H,s), 12.6(2H,*OH*,s) *m/z* (EI) 308.

3.11 Reaction of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione with thiols

3.11.1 With thiophenol

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.6 mmol) was added to a solution of sodium hydroxide (0.65g, 16 mmol) and thiophenol (1.73g, 16 mmol) in ethanol (25 ml), the mixture was heated under reflux for 24 h and then allowed to cool. The precipitate was filtered off to give a mixture of the starting material and benzene disulphide, mp 55°C (lit 59°C[32]), δ_H ($CDCl_3$) 7.3 (3H, m), 7.5 (2H, m).

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.6 mmol) was added to a solution of sodium hydroxide (0.65g, 16 mmol) and thiophenol (1.73g, 16 mmol) in propanol (25 ml). The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to give a mixture of the starting material and benzene disulphide, mp 55°C (lit 59°C[32]), δ_H ($CDCl_3$) 7.3 (3H, m), 7.5 (2H, m).

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.6 mmol) was added to a solution of sodium hydroxide (0.65g, 16 mmol) and thiophenol (1.73g, 16 mmol) in DMSO (25 ml). The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to give a mixture of the starting material and benzene disulphide, mp 55°C (lit 59°C[32]), δ_H ($CDCl_3$) 7.3 (3H, m), 7.5 (2H, m). The reaction was repeated varying concentration of sodium hydroxide and 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione but to no avail.

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.6 mmol) was added to a solution of sodium hydroxide (0.65g, 16 mmol) and thiophenol (1.73g, 16 mmol) in DMF (25 ml). The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to give a mixture of the starting material and benzene disulphide, mp 55°C (lit 59°C[32]), δ_H ($CDCl_3$) 7.3 (3H, m), 7.5 (2H, m).

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.6 mmol) was added to a solution of sodium hydroxide (0.65g, 16 mmol) and thiophenol (1.73g, 16 mmol) in DMF (25 ml) under a nitrogen atmosphere. The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to give the starting material. Identification was via TLC, using a mixture of 95% toluene and 5% chloroform (Rf= O.65).

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.6 mmol) was added to a solution of sodium carbonate (3.8g, 32 mmol) and thiophenol (1.73g, 16 mmol) in DMF (25 ml) under a nitrogen atmosphere. The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to give the starting material. Identification was via TLC, using a mixture of 95% toluene and 5% chloroform (Rf= O.65).

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.6 mmol) was added to a solution of potassium carbonate (4.6g, 32 mmol) and thiophenol (1.73g, 16 mmol) in DMF (25 ml). The solution was heated under reflux for 24 h and then allowed to cool.

The precipitate was then filtered off to give the starting material. Identification was via TLC, using a mixture of 95% toluene and 5% chloroform (Rf= O.65).

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.6 mmol) was added to a solution of potassium hydroxide (1.8g, 32 mmol) and thiophenol (1.73g, 16 mmol) in DMF (25 ml). The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to give the starting material. Identification was via TLC, using a mixture of 95% toluene and 5% chloroform (Rf= O.65).

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.2g, 0.8 mmol), thiophenol (0.14g), copper acetate[33] (0.40g) and sodium acetate was refluxed in amyl alcohol (25 ml) for 16 h. Amyl alcohol was removed by distillation to yield the starting material and benzene disulphide, mp 55°C (lit 59°C[32]), δ_H ($CDCl_3$) 7.3 (3H, m), 7.5 (2H, m).

3.11.2 With 2-methyl-2-propanethiol

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.6 mmol) was added to a solution of sodium hydroxide (0.65g, 16 mmol) and 2-methyl-2-propanethiol (1.50g, 16 mmol) in ethanol (25 ml). The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off, to give the starting material. The reaction was also repeated in propanol, DMF and DMSO to yield the starting materials. Identification was via TLC, using a mixture of 95% toluene and 5% chloroform (Rf= O.65).

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.6 mmol) was added to a solution of potassium hydroxide (0.90g, 16 mmol) and 2-methyl-2-propanethiol (1.50g, 16 mmol) in DMF (25 ml). The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to give the starting material. Identification was via TLC, using a mixture of 95% toluene and 5% chloroform (Rf= O.65).

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.6 mmol) was added to a solution of potassium carbonate (2.30g, 16 mmol) and 2-methyl-2-propanethiol (1.50g, 16 mmol) in DMF (25 ml). The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to yield the starting material. Identification was via TLC, using a mixture of 95% toluene and 5% chloroform (Rf= O.65).

3.11.3 With Potassium thiocyanate

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.6 mmol) was added to a solution of potassium thiocyanate (0.78g, 16 mmol) in ethanol (25 ml). The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to give the starting materials. The reaction was repeated using propanol, DMF and DMSO as the solvent to yield the starting material. Identification was via TLC, using a mixture of 95% toluene and 5% chloroform (Rf= O.65).

3.12 Synthesis of 1,4-dimethoxy-5,8-dichloroanthracene-9,10-dione

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.6 mmol) was added to a solution DMF (25 ml), dimethylsulphate (0.60g, 4.8 mmol) and potassium carbonate (1.00g, 7.2 mmol). The mixture under a nitrogen atmosphere was stirred and heated under reflux for 64 h. The hot solution was filtered and concentrated to give a dark solid, which was triturated with boiling petroleum ether. After standing the solid was collected by filtration to yield 1,4-dimethoxy-5,8-dichloroanthracene-9,10-dione (0.43g, 80%), mp 315°C (lit[16] 320-325°C), δ_H ($CDCl_3$) 3.9(6H, s), 7.1(2H, s), 7.5 (2H, s).

3.12.1 Reaction of thiols with 1,4-dimethoxy-5,8-dichloroanthracene-9,10-dione

3.12.2 With Thiophenol

1,4-Dimethoxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.5 mmol) was added to a solution of sodium hydroxide (0.65g, 16 mmol) and thiophenol(1.73g, 16 mmol) in ethanol (25 ml). The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to give the starting material. The reaction was also repeated in propanol, DMF and DMSO, to yield the starting material, mp 310°C, δ_H ($CDCl_3$) 3.9(6H, s), 7.1(2H, s), 7.5 (2H, s).

1,4-Dimethoxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.5 mmol) was added to a solution of sodium carbonate (0.65g, 16 mmol) and thiophenol (1.73g, 16 mmol) in DMF. The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to yield the starting material, mp 310°C, δ_H ($CDCl_3$) 3.9(6H, s), 7.1(2H, s), 7.5 (2H, s).

1,4-Dimethoxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.5 mmol) was added to a solution of potassium hydroxide (0.90g, 16 mmol) and thiophenol (1.73g, 16 mmol) in DMF. The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to yield the starting material, mp 310°C, δ_H ($CDCl_3$) 3.9(6H, s), 7.1(2H, s), 7.5 (2H, s).

1,4-Dimethoxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.5 mmol) was added to a solution of potassium carbonate (2.30g, 16 mmol) and thiophenol (1.73g, 16 mmol) in DMF. The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to yield the starting material, mp 310°C, δ_H ($CDCl_3$) 3.9(6H, s), 7.1(2H, s), 7.5 (2H, s).

3.12.3 With Potassium thiocyanate

1,4-Dimethoxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.5 mmol) was added to a solution potassium thiocyanate (0.78g, 16 mmol) in ethanol (25 ml). The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to give the starting material. The reaction was repeated using propanol, DMF and DMSO, to yield the starting materials, mp 310°C, δ_H ($CDCl_3$) 3.9(6H, s), 7.1(2H, s) 7.5, (2H, s).

3.12.4 With 2-methyl-2-propanethiol

1,4-Dimethoxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.5 mmol) was added to a solution of sodium hydroxide (0.65g, 16 mmol) and 2-methyl-2-propanethiol (1.50g, 16 mmol) in ethanol (25 ml). The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to give the starting material. The

reaction was also repeated in propanol, DMF and DMSO to yield the starting materials, mp 310°C, δ_H ($CDCl_3$) 3.9(6H, s), 7.1(2H, s), 7.5 (2H, s).

1,4-Dimethoxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.5 mmol) was added to a solution of potassium hydroxide (0.90g 16 mmol) and 2-methyl-2-propanethiol (1.50g, 16 mmol) in DMF. The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to give the starting material, mp 310°C, δ_H ($CDCl_3$) 3.9(6H, s), 7.1(2H, s), 7.5 (2H, s).

1,4-Dimethoxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.5 mmol) was added to a solution of potassium carbonate (2.30g, 16 mmol) and 2-methyl-2-propanethiol (1.50g, 16 mmol) in DMF. The solution was heated under reflux for 24 h and then allowed to cool. The precipitate was then filtered off to give the starting material, mp 310°C, δ_H ($CDCl_3$) 3.9(6H, s), 7.1(2H, s), 7.5 (2H, s).

3.13 Reduction of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione

3.13.1 Tin in acetic acid 34

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (1.00g, 3.3 mmol) was added to a mixture of tin (3.00g, 24.4 mmol) and HCl (70 ml) in acetic acid (50 ml). The mixture was heated to 90-95°C for 24 hrs. The solution was allowed to cool and the precipitate was filtered off, the solid was washed with water and dried in vacuo to yield 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (0.65, 64%), mp 147°C, δ_H ($CDCl_3$) 3.0(4H,s), 7.6(2H,s), 14.5(2H,*OH*,s).

3.13.2 Sodium dithionite [35 36]

1,4-Dihydroxy-5,8-dichloroanthracene-9,10-dione (1.00g, 3.3 mmol) was added to a solution of sodium carbonate (1.4g, 13.2 mmol) and water (125 ml). The solution was heated until boiling, to which was added sodium dithionite (1.7g, 9.8 mmol). An additional quantity of sodium dithionite (0.50g, 2.9 mmol) was added to the solution to complete the reduction. The precipitate was filtered off, washed with dilute acetic acid until alkali free and dried in vacuo to yield 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (0.90g 96%), mp 145°C, (lit[36]150°C), δ_H ($CDCl_3$) 3.0(4H,s), 7.6(2H,s), 14.5(2H,*OH*,s).

3.14 Amination of 9,10-dihydroxy-5,8-dichloro-2,3-dihydroanthracene-1,4-dione

3.14.1 Using Aniline

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of aniline (4.10g, 44 mmol) and was heated for 4 h at 90-95°C. The solution was allowed to cool, the precipitate was filtered off, washed with ethanol and dried to yield the starting material 9,10-dihydroxy-5,8-dichloro-2,3-dihydroanthracene-1,4-dione. Identification was via TLC using 95% toluene & 5% chloroform (Rf= 0.55).

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of aniline (4.10g, 44 mmol) in ethanol (25 ml) and was heated for 4 h at 90-95°C. The solution was allowed to cool and the precipitate was filtered off. The solid was washed with ethanol and dried to yield the starting material 9,10-dihydroxy-

5,8-dichloro-2,3-dihydro-anthracene-1,4-dione. Identification was via TLC using 95% toluene & 5% chloroform (Rf= 0.55).

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of aniline (3.00g, 32 mmol) and boric acid (0.40g, 6.4 mmol) and was heated for 4 h at 90-95°C. The solution was allowed to cool, the precipitate filtered off, and purified by column chromatography using an eluent of toluene 95% and chloroform 5% to yield the 1,4-bis(phenylamino)-5,8-dichloro-anthracene-9,10-dione (0.35g, 24%), mp 230°C (lit[34] 234-5°C), δ_H ($CDCl_3$) 7.00-7.30 (10H m), 7.40(2H s), 7.50(2H s), 11.40 (2H *NH* s), δ_c 182.26, 142.77, 139.97, 136.43, 133.60, 133.26,129.92, 126.51, 125.30, 123.92, 113.42

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of aniline (3.00g, 32 mmol) and boric acid (0.40g, 6.4 mmol) in ethanol (30 ml) and was heated for 4 h at 90-95°C. The solution was allowed to cool, the precipitate was filtered off and purified by column chromatography using a eluent of toluene 95% and chloroform 5% to yield the 1,4-bis(phenylamino)-5,8-dichloro-anthracene-9,10-dione (0.75g, 51%), mp 225-8°C (lit[34] 234-5°C), δ_H ($CDCl_3$) 7.00-7.30 (10H m), 7.40(2H s), 7.50(2H s), 11.40 (2H *NH* s).

3.14.2 Using Isobutylamine

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of isobutylamine (2.40g, 32 mmol) and was heated for 1 h at 50-5°C. The solution was allowed to cool to which was added petroleum ether 40-60 (25 ml). The mixture was stirred for 1 h and the solid was filtered off, purified by column

chromatography using an eluent of toluene 95% and chloroform 5% to yield 1,4-bis(isobutylamino)-5,8-dichloro-anthracene-9,10-dione(0.30g, 22%), mp 152 °C, δ_H ($CDCl_3$) 1.00(12H, d), 2.00 (2H, m), 3.10(4H, t), 7.10(2H,s), 7.50(2H,s), 10.40 (2H, *NH* t), δ_c 180.80, 145.75, 135.69, 133.44, 133.30, 123.70, 110.68, 61.55, 29.09, 20.92.

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of isobutylamine (1.00g, 17 mmol) in ethanol (25 ml) and was heated for 1 h at 50-5°C. The solution was allowed to cool to which was added petroleum ether 40-60 (25 ml). The mixture was stirred for overnight and the solid was filtered off, purified by column chromatography using an eluent of toluene 95% and chloroform 95% to yield 1,4-bis(isobutylamino)-5,8-dichloro-anthracene-9,10-dione (0.70g, 52%), mp 154°C, δ_H ($CDCl_3$) 1.00(12H, d), 2.00 (2H, m) 3.10 (4H, t), 7.10 (2H,s), 7.50 (2H,s), 10.40 (2H, *NH* t), δ_c 180.80, 145.75, 135.69, 133.44, 113.30, 123.70, 110.68, 61.55, 29.09, 20.92.

3.14.3 Using 2-Aminoethanol

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of 2-aminoethanol (2.10g, 34 mmol) and was heated for 4 h at 50-55°C. The solution was allowed to cool to which was added petroleum ether 40-60 (25 ml). The mixture was stirred for 1 h and the solid was filtered off, the solid was purified via column chromatography using an eluent of toluene 95% and chloroform 5% to yield 1,4-bis[2-(hydroxyethyl)amino]-5,8-dichloro-anthracene-9,10-dione (0.40g, 31%), mp

190°C, δ_H ($CDCl_3$) 3.40(4H, q), 3.60 (4H,t) 4.90(2H, *OH* s), 7.35 (2H,s) 7.60(2H,s), 10.30 (2H, *NH* t).

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of 2-aminoethanol (1.00g, 17 mmol) in ethanol (25 ml) and was heated for 1 h at 50-5°C. The solution was allowed to cool to which was added petroleum ether 40-60 (25 ml). The mixture was stirred for overnight and the solid was filtered off and was recrystallised from ethanol to yield 1,4-bis[2-(hydroxyethyl)amino]-5,8-dichloroanthracene-9,10-dione(0.8g, 64%), mp 197°C, δ_H ($CDCl_3$) 3.40(2H,t), 3.60 (2H, q) 4.90 (2H, *OH* s), 7.35 (4H, s) 7.60(4H, s), 10.30 (2H, *NH* t).

3.14.4 Using Propylamine

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of propylamine (2g, 34 mmol) and was heated for 1 h at 50°C. The solution was allowed to cool to which was added petroleum ether 40-60 (25 ml). The mixture was stirred for 1 h and the precipitate was filtered off. The solid was purified via column chromatography using an eluent of toluene 95% and chloroform 5%, to yield 1,4-bis(propylamino)-5,8-dichloroanthracene-9,10-dione (0.40g, 32%), mp 150°C, δ_H ($CDCl_3$) 1.00 (6H,t), 1.50 (4H,m) 3.30 (4H, q), 7.10 (2H,s) 7.40 (2H,s), 10.30 (2H, *NH* t).

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of propylamine (1.00g, 17 mmol) in ethanol (25 ml) and was heated for 1 h at 50-5°C. The solution was allowed to cool to which was added petroleum ether 40-60 (25 ml) and was stirred for overnight. The solid was filtered off and purified via

column chromatography using an eluent of toluene 95% and chloroform 5% to yield 1,4-bis(propylamino)-5,8-dichloroanthracene-9,10-dione (0.6g, 48%), mp 150°C, δ_H ($CDCl_3$) 1.00 (6H,t), 1.50 (4H,m) 3.30 (4H, q), 7.10 (2H,s) 7.40 (2H,s), 10.30 (2H, *NH* t).

3.14.5 Using Methylamine

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of 20% methylamine in water (4.96g, 32 mmol) and was heated for 1 h at 50-5°C. The solution was allowed to cool and the solid was collected by filtration. The solid was washed with water (50 ml) and purified by column chromatography using an eluent of toluene 95% and chloroform 5% to yield 1,4-bis(methylamino)-5,8-dichloroanthracene-9,10-dione (0.50g, 47%), mp 145 °C, δ_H ($CDCl_3$) 3.00 (6H, d), 7.15(2H,s) 7.45(2H,s), 10.10 (2H, *NH*).

3.14.6 Using N,N-Dimethylethylenediamine

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to N,N-dimethylethylenediamine (2.8g, 32 mmol) and was heated for 1 h at 50-5°C. The solution was allowed to cool, to which was added petroleum ether 40-60 (25 ml). The mixture was refrigerated for 7 days to yield a tar like solid, further attempts to purify the mixture where unsuccessful

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of N,N-dimethylethylenediamine (2.8g, 16 mmol) in ethanol (25 ml) and was heated for 2 h at 50-5°C. The solution was allowed to cool to which was added petroleum ether 40-60 (25 ml). The mixture was refrigerated, the solid was filtered off

and added to a solution of diethylether (25 ml) and methanol (25 ml) saturated with HCl (HCl gas was generated by adding conc. hydrochloric acid 50 ml, dropwise to concentrated sulphuric acid 50 ml, and the gas evolved was bubbled through the solution). The solid was purified using column chromatography via column chromatography with an eluent of 10% methanol and 90% chloroform to yield 1,4-bis{[2-(dimethylamino)ethyl]amino}-5,8-dichloroanthracene-9,10-dione[37] (0.40g, 29%), mp 255°C, δ_H (DMSO d^6) 3.20 (12H, s), 3.30 (4H, t), 3.50 (4H, q), 7.35 (2H,s) 7.65 (2H,s), 10.10 (2H, *NH* t), δ_c 179.91, 144.45,136.03, 132.23, 132.09, 124.66, 109.78, 55.55, 51.74, 46.79.

3.14.7 Using N,N-Diethylethylenediamine

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to N,N-diethylethylenediamine (2.8g, 32 mmol) and heated for 1 h at 50-5°C. The solution was allowed to cool to which was added petroleum ether 40-60 (25 ml) the mixture was refrigerated for 7 days to yield a tar like solid, further attempts to purify the mixture was unsuccessful.

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of N,N-diethylethylenediamine (2.8g, 16 mmol) in ethanol (25 ml) and was heated for 2 h at 50-5°C. The solution was allowed to cool to which was added petroleum ether 40-60 (25 ml). The mixture was refrigerated, the solid was filtered off and added to a solution of diethylether (25 ml) and methanol (25 ml) saturated with HCl (HCl gas was generated by adding conc. hydrochloric acid 50 ml, dropwise to concentrated sulphuric acid 50 ml, and the gas evolved was bubbled through the

solution). The solid was purified using column chromatography via column chromatography with an eluent of 10% methanol and 90% chloroform to yield 1,4-bis{[2-(diethylamino)ethyl]amino}-5,8-dichloroanthracene-9,10-dione. HCl (0.30g, 22%), mp 265°C, δ_H (DMSO d^6) 1.25 (12H, t), 3.20 (8H, q), 3.40 (4H, t) 3.90 (4H, q), 7.60 (2H, s) 7.80 (2H, s), 9.90 (2H, *NH* t), 10.90 (2H, *HCl*), δ_c 180.51, 144.04,136.61, 132.54, 132.26, 124.20, 111.04, 49.94, 46.75, 37.36,8.69

3.14.8 Using 2-[2-(aminoethyl)amino]ethanol

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (1.00g, 3.2 mmol) was added to a solution of 2-[2-(aminoethyl)amino]ethanol (3.3g, 32 mmol). The solution was heated for 1 h at 50-5°C and then allowed to cool, to which was added petroleum ether 40-60 (25 ml). The mixture was refrigerated for 7 days to yield a tar like solid, further attempts to purify the mixture were unsuccessful. The reaction was repeated altering the molar ratios of reactants, the reaction time and the solvent employed i.e. methanol, ethanol, propanol, butanol and 2-methoxyethanol but only yielded tarry solid. Purification of the tarry solid was attempted via column chromatography using eluting solvent systems of 90% chloroform & 10% methanol or 70% THF, 20% acetic acid & 10% water but unsuccessful.

3.15 Reaction of 1,4-bis(phenylamino) -5,8-dichloroanthracene-9,10-dione

3.15.1 With Thiophenol

1,4-Bis(phenylamino) -5,8-dichloroanthracene-9,10-dione (0.50 g, 1.1 mmol) was added to a solution of thiophenol (1.20g, 11 mmol) and potassium hydroxide (0.62g, 11 mmol) in DMF (25 ml) and was heated under reflux for 2h. The DMF was removed under vacuum distillation, the solid was washed with 10% potassium hydroxide solution (50 ml) and then methanol. The solid was purified via column chromatography using an eluent of 90% toluene and 10% chloroform to yield 1,4-bis(phenylamino)-5,8-bis(phenylsulfanyl)anthracene-9,10-dione[38], (0.40g, 60%), mp 225°C, δ_H ($CDCl_3$) 6.65 (2H s), 7.00-7.50 (22H m), 11.85 (2H *NH* s), δ_c 184.56, 143.46, 141.60, 137.44, 133.51, 131.34, 130.32, 130.29, 129.85, 129.49, 129.43, 127.91, 125.03, 124.38, 112.25.

3.15.2 With 2-Ethanethiol

1,4-Bis(phenylamino) -5,8-dichloroanthracene-9,10-dione (0.50g, 1.1 mmol) was added to a solution of 2-ethanethiol (0.68g, 11 mmol) and potassium hydroxide (0.62g, 11 mmol) in DMF (25 ml) and was heated under reflux for 2h. The DMF was removed under vacuum distillation, the solid was washed with 10% potassium hydroxide solution (50 ml) and then methanol. The solid was purified via column chromatography using an eluent of 90% toluene and 10% chloroform to yield (0.30g, 52%) 1,4-bis(phenylamino)-5,8-bis(ethylsulfanyl) anthracene-9,10-dione, mp 215°C, δ_H ($CDCl_3$), 1.35 (6H, t), 2.90 (4H, q), 7.00-7.50 (14H, m), 11.75 (2H, *NH*, s), δ_c 184.74, 140.26, 139.77, 132.77,

132.22, 129.89, 129.79, 128.50, 124.85, 124.79, 112.43, 26.79, 13.23. m/z calculated 510.1436 found 510.1434

3.15.3 With Potassium thiocyanate

1,4-Bis(phenylamino) -5,8-dichloroanthracene-9,10-dione (0.50g, 1.1 mmol) was added to a solution of potassium thiocyanate (1.10g, 11 mmol) in DMF (25 ml) and was heated under reflux for 48 h. The DMF was removed under vacuum distillation, the solid was washed with 10% potassium hydroxide solution (50 ml) to yield the starting material, identification was via TLC using 95% methanol and 5% chloroform(Rf=0.88)

3.15.4 With 2-methyl-2-propanethiol

1,4-Bis(phenylamino) -5,8-dichloroanthracene-9,10-dione (0.50g, 1.1 mmol) was added to a solution of 2-methyl-propanethiol (0.99g, 11 mmol) and potassium hydroxide (0.62g, 11 mmol) in DMF (25 ml) and was heated under reflux for 24h. The DMF was then removed under vacuum distillation, the solid was washed with 10% potassium hydroxide solution (50 ml) and then methanol to yield the starting material, identification was via TLC using 95% methanol and 5% chloroform(Rf=0.88).

3.16 Reaction of 1,4-bis(propylamino)-5,8-dichloroanthracene-9,10-dione

3.16.1 With Thiophenol

1,4- Bis(propylamino)-5,8-dichloro-anthracene-9,10-dione (0.43g, 1.1 mmol) was added to a solution of thiophenol (1.20g, 11 mmol) and potassium hydroxide (0.62g, 11 mmol)

in ethanol (25 ml). The solution was heated under reflux for 2h and the ethanol was removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml) and then methanol. The solid was purified via column chromatography using an eluent of 90% toluene and 10% chloroform to yield 1,4-bis bis(propylamino)-5,8-bis(phenylsulfanyl)anthracene-9,10-dione (0.25g, 41%), mp 240°C, δ_H ($CDCl_3$) 1.00 (6H, t), 1.8 (4H, m), 3.30(4H, q), 6.60 (2H, s) 7.40 (12H,m), 10.50 (2H, NH, t), *m/z* (EI) 538.3.

1,4- Bis(propylamino)-5,8-dichloro-anthracene-9,10-dione (0.43g, 1.1 mmol) was added to a solution of thiophenol (1.20g, 11 mmol) and potassium hydroxide (0.62g, 11 mmol) in DMF (25 ml). The solution was heated under reflux for 2h and then the DMF was removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml) and then methanol. The solid was purified via column chromatography using an eluent of 90% toluene and 10% chloroform to yield 1,4-bis bis(propylamino)-5,8-bis(phenylsulfanyl)anthracene-9,10-dione (0.40g, 66%), mp 240°C, δ_H ($CDCl_3$) 1.00 (6H, t), 1.80 (4H, m), 3.30 (4H, q), 6.60 (2H, s) 7.40 (12H,m), 10.50 (2H, NH, t), *m/z* (EI) 538.3.

3.16.2 With Ethanethiol

1,4- Bis(propylamino) -5,8-dichloroanthracene-9,10-dione (0.43g, 1.1 mmol) was added to a solution of ethanethiol (0.68g, 11 mmol) and potassium hydroxide (0.62g, 11 mmol) in DMF (25 ml). The solution was heated under reflux for 2h and then the DMF was removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml) and then methanol. The solid was purified via column

chromatography using an eluent of 90% toluene and 10% chloroform to yield 1,4-bis(propylamino) 5,8-bis(ethylsulfanyl) anthracene-9,10-dione (0.30g, 62%), mp 235°C, δ_H ($CDCl_3$) 1.00 (6H, t) 1.30 (6H, t), 1.70 (4H, m), 2.80 (4H, q), 3.20 (4H, q), 6.90 (2H, s), 7.25 (2H, s), 10.30 (2H, *NH*, t).

1,4- Bis(propylamino) -5,8-dichloroanthracene-9,10-dione (0.43g, 1.1 mmol) was added to a solution of ethanethiol (0.68g, 11 mmol) and potassium hydroxide (0.62g, 11 mmol) in DMF (25 ml). The solution was heated under reflux for 24h and then the DMF was removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml) and then methanol. The solid was purified via column chromatography using an eluent of 90% toluene and 10% chloroform to yield 1,4-diamino-5,8-bis(ethylsulfanyl)anthracene-9,10-dione (0.10g, 22%), mp 235°C, δ_H (DMSO) 1.40 (6H, t), 3.00 (4H, q), 7.20 (2H s), 7.65 (2H, s), 8.20 (4H, s,) *m/z* (EI) 358.1 and 1-(propylamino)-4-amino-5,8-bis(ethylsulfanyl) anthracene-9,10-dione, (0.12g, 25%), mp 210 °C, δ_H($CDCl_3$) 1.00 (3H, t), 1.40 (6H, t), 1.70 (2H,m), 2.9 (4H, m), 3.3 (2H, q), 6.8 (2H, s) 7.00 (2H, m), 7.5 (2H, m), 10.30 (1H, t), *m/z* (EI) 400.2.

3.16.3 With Potassium thiocyanate

1,4- Bis(propylamino) -5,8-dichloroanthracene-9,10-dione (0.43g, 1.1 mmol) was added to a solution of potassium thiocyanate (1.10g, 11 mmol) in DMF (25 ml). The solution was heated under reflux for 2h and then the DMF was removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml), to yield the starting material; identification was via TLC using 95% methanol and 5% chloroform.

1,4- Bis(propylamino) -5,8-dichloroanthracene-9,10-dione (0.43g, 1.1 mmol) was added to a solution of potassium thiocyanate (1.10g, 11 mmol) in DMF (25 ml). The solution was heated under reflux for 24 h and then the DMF was removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml) and purified by column chromatography using an eluent of 10% methanol and 90% chloroform to yield 1,4-diamino-5,8-dichloro-anthracene-9,10-dione (0.08g, 25%), mp 270°C, (lit[31] 280°C), *m/z* (EI) 306.1, δ_H ($CDCl_3$) 6.75 (4H, s *NH_2*), 6.80 (2H, s), 7.50 (2H, s).

1,4- Bis(propylamino) -5,8-dichloroanthracene-9,10-dione (0.43g, 1.1 mmol) in DMF (25 ml) was heated under reflux for 24 h. The DMF was removed under vacuum distillation and the resulting solid was purified by column chromatography using an eluent of 10% methanol and 90% chloroform to yield 1,4-diamino-5,8-dichloro-anthracene-9,10-dione (0.08g, 25%), mp 270°C, (lit[31] 280°C), *m/z* (EI) 306.1, δ_H ($CDCl_3$) 6.75 (4H, s *NH_2*), 6.80 (2H, s), 7.50 (2H, s).

1,4- Bis(propylamino) -5,8-dichloroanthracene-9,10-dione (0.43g, 1.1 mmol) with 30% hydrogen peroxide solution (10 ml) in DMF (25 ml) was heated under reflux for 2 h. The DMF was removed under vacuum distillation and the resulting solid was purified by column chromatography using an eluent of 10% methanol and 90% chloroform to yield 1,4-diamino-5,8-dichloro-anthracene-9,10-dione (0.10g, 32%), mp 270°C, (lit[31] 280°C), *m/z* (EI) 306.1, δ_H ($CDCl_3$) 6.75 (4H, s *NH_2*), 6.80 (2H, s), 7.50 (2H, s).

1,4- Bis(propylamino) -5,8-dichloroanthracene-9,10-dione (0.43g, 1.1 mmol) in DMF (25 ml) previously purged with nitrogen. The solution was heated under reflux for 48 h and nitrogen was bubbled through. The DMF was removed under vacuum distillation to yield the starting material. Identification was via TLC using 95% methanol and 5% chloroform.

3.17 Reaction of 1,4-bis(isobutylamino)-5,8-dichloro-9,10-anthracenedione

3.17.1 With Thiophenol

1,4- Bis(isobutylamino)-5,8-dichloro-anthracene-9,10-dione (0.43g, 1.1 mmol) was added to a solution of thiophenol (1.20g, 11 mmol) and potassium hydroxide (0.62g, 11 mmol) in DMF (25 ml). The solution was heated under reflux for 2h and then the DMF was removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml) and then methanol. The solid was purified via column chromatography using an eluent of 10% methanol and 90% chloroform to yield 1,4-bis bis(isobutylamino)-5,8-bis(phenylsulfanyl)anthracene-9,10-dione (0.22g, 37%), mp 240°C, δ_H ($CDCl_3$) 1.00 (12H, d), 2.10 (2H, m), 3.30 (4H, t), 6.60 (2H, s) 7.50 (12H, m), 10.60 (2H, NH, t).

3.17.2 With Ethanethiol

1,4- Bis(isobutylamino)-5,8-dichloro-anthracene-9,10-dione (0.43g, 1.1 mmol) was added to a solution of ethanethiol (0.68g, 11 mmol) and potassium hydroxide (0.62g, 11 mmol) in DMF (25 ml). The solution was heated under reflux for 2h and then the DMF was removed under vacuum distillation. The solid was washed with 10% potassium

hydroxide solution (50 ml) and then methanol. The solid was purified via column chromatography using an eluent of 90% toluene and 10% chloroform to yield 1,4-bis(isobutylamino) 5,8-bis(ethylsulfanyl) anthracene-9,10-dione (0.2g 41%), mp 230 °C, δ_H 0.95 (12H, d), 1.30 (6H, t), 1.90 (2H, m), 2.80 (4H, q), 3.05(4H, t), 6.95 (2H, s), 7.30 (2H,s), 10.40, (2H, t, *NH*), δ_c 182.17, 137.02, 131.11, 128.23, 127.34, 124.48, 108.4, 49.63, 29.79, 28.79, 19.53, 11.80.

3.17.3 With Potassium thiocyanate

1,4- Bis(isobutylamino)-5,8-dichloro-anthracene-9,10-dione (0.43g, 1.1 mmol) was added to a solution of potassium thiocyanate (1.10g, 11 mmol) in DMF (25 ml). The solution was heated under reflux for 2h and then the DMF was removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml) to yield the starting material, identification was via TLC using 95% methanol and 5% chloroform(Rf =0.88).

1,4- Bis(isobutylamino)-5,8-dichloro-anthracene-9,10-dione (0.43g, 1.1 mmol) was added to a solution of potassium thiocyanate (1.10g, 11 mmol) in DMF (25 ml). The solution was heated under reflux for 24 h and then the DMF was removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml) to yield 1,4-diamino-5,8-dichloro-anthracene-9,10-dione (0.05 g, 13%), mp 270°C, (lit[31] 280°C), *m/z* (EI) 306.1.

3.18 Reaction of 1,4-bis[2-(hydroxyethyl)amino]-5,8-dichloro-9,10-anthracenedione

3.18.1 With Thiophenol

1,4- Bis[2-(hydroxyethyl)amino]-5,8-dichloro-anthracene-9,10-dione (0.43g, 1.1 mmol) was added to a solution of thiophenol (1.20g, 11 mmol) and potassium hydroxide (0.62g, 11 mmol) in DMF (25 ml). The solution was heated under reflux for 2h and then the DMF was removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml) and then methanol. The solid was purified via column chromatography to yield 1,4-bis bis[2-(hydroxyethyl)amino]-5,8-bis(phenylsulfanyl)anthracene-9,10-dione (0.40g, 67%), mp 260°C, δ_H (DMSO) 3.40 (4H, q), 3.75 (4H t), 5.10 (2H, *OH* s), 6.70 (2H s), 7.50 (12H m), 10.50 (2H *NH* s), δ_c 181.64, 145.64, 139.88, 135.95,133.33, 130.50, 130.45, 129.87, 129.17, 124.56, 108.77, 60.27, 45.19.

3.18.2 With Ethanethiol

1,4-Bis[2-(hydroxyethyl)amino]-5,8-dichloro-anthracene-9,10-dione(0.43g, 1.1 mmol) was added to a solution of ethanethiol (0.68g, 11 mmol) and potassium hydroxide (0.62g, 11 mmol) in DMF (25 ml). The solution was heated under reflux for 2h and the DMF was then removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml) and then methanol. The solid was purified via column chromatography using an eluent of 90% toluene and 10% chloroform to yield 1,4-bis[2-(hydroxyethyl)amino]-5,8-bis(ethylsulfanyl) anthracene-9,10-dione (0.25g,

51%), mp 254°C, δ_H ($CDCl_3$) 1.10 (6H, t), 2.70 (4H, q), 3.30 (4H, q), 3.50 (4H, t), 7.20 (2H, s), 7.4 (2H, s), 10.10 (2H, *NH), m/z* (EI) 446.2.

1,4-Bis[2-(hydroxyethyl)amino]-5,8-dichloro-anthracene-9,10-dione(0.43g, 1.1 mmol) was added to a solution of ethanethiol (0.68g, 11 mmol) and potassium hydroxide (0.62g, 11 mmol) in DMF (25 ml). The solution was heated under reflux for 24h and the DMF was then removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml) and then methanol. The solid was purified via column chromatography using an eluent of 90% toluene and 10% chloroform to yield 1,4-diamino-5,8- bis(ethylsulfanyl) anthracene-9,10-dione (0.13g, 25%), mp 256°C, δ_H (DMSO), 1.40 (6H, t), 3.00 (4H, q), 7.20 (2H s), 7.65 (2H, s), 8.20 (4H, s,), *m/z* (EI) 358.1.

3.18.3 With Potassium thiocyanate

1,4-Bis[2-(hydroxyethyl)amino]-5,8-dichloro-anthracene-9,10-dione(0.43g, 1.1 mmol) was added to a solution of potassium thiocyanate (1.10g, 11 mmol) in DMF (25 ml). The solution was heated under reflux for 24 h and was then the DMF was removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml) and purified by column chromatography using an eluent of 10% methanol and 90% chloroform to yield 1,4-diamino-5,8-dichloro-anthracene-9,10-dione (0.10g, 27%), mp 270°C, (lit[31] 280°C), *m/z* (EI) 306.1.

1,4-Bis[2-(hydroxyethyl)amino]-5,8-dichloro-anthracene-9,10-dione(0.43g, 1.1 mmol) in DMF (25 ml) and was heated under reflux for 24 h. The DMF was then removed under vacuum distillation and the solid was purified by column chromatography using an eluent

of 10% methanol and 90% chloroform to yield 1,4-diamino-5,8-dichloro-anthracene-9,10-dione (0.12g, 31%), mp 270°C, (lit[31] 280°C), *m/z* (EI) 306.1.

1,4-Bis[2-(hydroxyethyl)amino]-5,8-dichloro-anthracene-9,10-dione(0.43g, 1.1 mmol) with 30% hydrogen peroxide solution (10 ml) in DMF (25 ml)and was heated under reflux for 2 h. The DMF was then removed under vacuum distillation and the solid was purified by column chromatography using an eluent of 10% methanol and 90% chloroform to yield 1,4-diamino-5,8-dichloro-anthracene-9,10-dione (0.12g, 31%), mp 270°C, (lit[31] 280°C), *m/z* (EI) 306.1.

1,4-Bis[2-(hydroxyethyl)amino]-5,8-dichloro-anthracene-9,10-dione(0.43g, 1.1 mmol) was added to DMF (25 ml) previously purged with nitrogen and was heated under reflux for 24h under a nitrogen atmosphere. The DMF was then removed by vacuum distillation to yield the starting material; identification was via TLC using 95% chloroform and 5% methanol.

3.19 Reaction of 1,4-bis{[2-(dimethylamino)ethyl]amino}-5,8-dichloroanthracene-9,10-dione and 1,4-bis{[2-(diethylamino)ethyl]amino]-5,8-dichloro-anthracene-9,10-dione

1,4-Bis{[2-(dimethylamino)ethyl]amino]-5,8-dichloro-anthracene-9,10-dione (0.43g, 1.1 mmol) was added to a solution of thiophenol (1.20g, 11 mmol) and potassium hydroxide (0.62g, 11 mmol) in DMF (25 ml) and was heated under reflux for 2h. The DMF was then removed under vacuum distillation. The solid was washed with 10% potassium hydroxide solution (50 ml) to yield the starting material, identification was via TLC using

an eluent of 10% methanol and 90% chloroform (Rf =0.40). The reaction was repeated with 2-ethanethiol, and potassium thiocyanate but to no avail. Prolonged reaction times only led to the previously isolated decomposition product 1,4-diamino-5,8-dichloroanthracene-9,10-dione.

The above reaction was also repeated with 1,4-bis{[2-(diethylamino)ethyl]amino]-5,8-dichloro-anthracene-9,10-dione and yielded the same outcome.

3.20 Synthesis of 1,4-bis(amino)anthracene-9,10-diones

2,3-Dihydro-9,10-dihydroxyanthracene-1,4-dione (1.00g, 4.1 mmol) was added to a solution of 2-aminoethanol (1.20g, 20 mmol) in ethanol (25 ml). The solution was heated for 1 h at 50-5°C. The solution was allowed to cool, the solid was filtered off and recrystallised from ethanol to yield 1,4-bis[2-(hydroxyethyl)amino]anthracene-9,10-dione (0.6g, 45%), mp 225 °C (lit[39] 237°C).

2,3-Dihydro-9,10-dihydroxyanthracene-1,4-dione (1.00g, 4.1 mmol) was added to a solution of benzylamine (1.90g, 20 mmol) in ethanol (25 ml). The solution was heated for 1 h at 50-5°C. The solution was allowed to cool, the solid was filtered off and recrystallised from ethanol to yield 1,4-bis(benzylamino)anthracene-9,10-dione (0.8, 49%), mp 195°C,(lit[40] 205°C).

2,3-Dihydro-9,10-dihydroxyanthracene-1,4-dione (1.00g, 4.1 mmol) was added to a solution of propylamine (1.20g, 20 mmol) in ethanol (25 ml). The solution was heated for 1 h at 50-5°C. The solution was allowed to cool, the solid was filtered off and recrystallised from ethanol to yield 1,4-bis(propylamino)anthracene-9,10-dione (0.6g, 45%), mp 127°C, (lit[41] 132°C).

1,4-Bis[2-(hydroxyethyl)amino]anthracene-9,10-dione (0.50g, 2.1 mmol) was heated under reflux in DMF (25 ml) for 24 hours. The DMF was then removed under vacuum distillation to and purified by column chromatography using an eluent of 10% methanol and 90% chloroform to yield 1,4-diaminoanthracene-9,10-dione (0.10g, 20%) mp 250°C (lit[42] 265°C), $\delta_H(CDCl_3)$ 6.7 (2H, s), 7.0 (4H, NH), 7.6 (2H, m), 8.4, (2H, m).

The above reaction was repeated with 1,4-bis(propylamino)anthracene-9,10-dione and 1,4-bis(benzylamino)anthracene-9,10-dione to yield 1,4-diaminoanthracene-9,10-dione.

3.21 Synthesis of 1,4-diamino-5,8-dichloroanthracene-9,10-dione

3.21.1 Amination of 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-9,10-dione

2,3-Dihydro-9,10-dihydroxy-5,8-dichloroanthracene-9,10-dione (0.50g, 1.5 mmol) was dissolved in ethanol 25 ml, the mixture was heated to 50°C and ammonia gas was bubbled through over 24h. The solution was cooled and the precipitate was filtered off to yield the starting material, identification was performed using TLC with an eluent of 5% chloroform and 95% toluene (Rf =0.50).

3.22 Chlorination of 1,4-diaminoanthracene-9,10-diones

1,4-Diaminoanthracene-9,10-dione (5.0g, 21 mmol) was added to a mixture of boric acid (2.6g, 4.2 mmol), 65% oleum (80g), chlorosulphonic acid (80 ml, 0.3 mol) and iodine (1.00g, 3.9 mmol). The mixture was heated to 70°C for 24 h. The mixture was allowed to cool and poured onto ice (1000g). The precipitate was collected by filtration and washed with water. The solid was recrystallised from DMF (150 ml) to give a mixture of chlorinated products, *m/z* (EI) 306.1, 340.1, 375.0.

3.23 Instrumentation

^{1}H NMR were recorded on a Bruker AC spectrometer at 400 MHz. All chemical shifts in parts per million (δ) relative to tetramethylsilane in deuturated dimethylsulphoxide (DMSO) or Chloroform ($CDCl_3$). Mass spectra were recorded using the EPSRC Mass spectrometry centre using a VG analytical Quatro II triple quadrupole mass spectrometer. The accurate masses were obtained on a Finngan MAT 900 XL using a perflurotributyl amine as the reference compound for electron ionisation and polyethyleneimine for chemical ionisations. Melting points were obtained on an electrothermal digital melting point apparatus.

3.24 References

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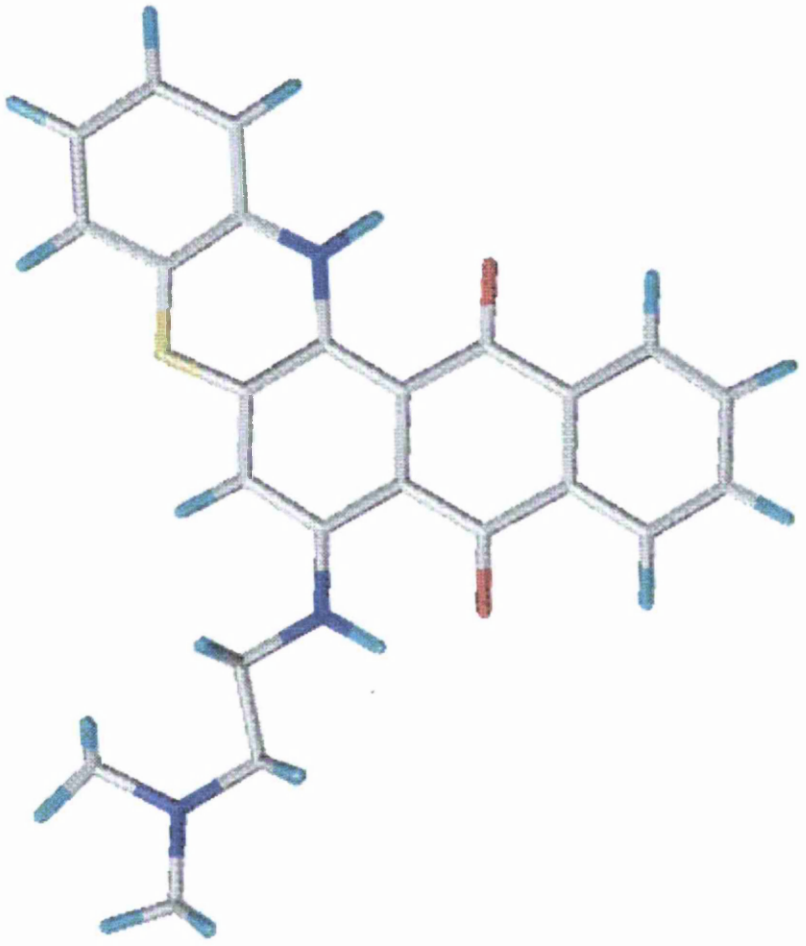
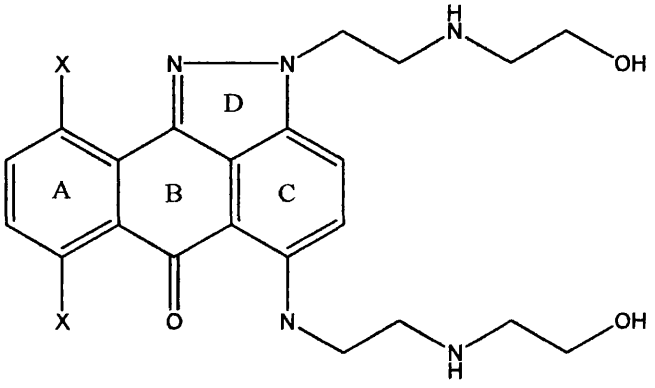
CHAPTER FOUR



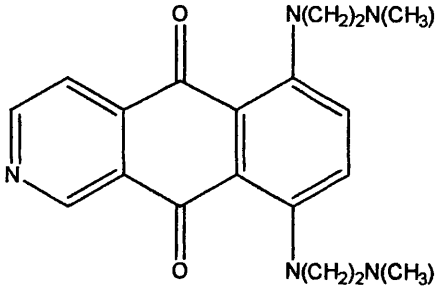
SYNTHESIS OF 7-AMINOALKYL-14H-NAPTHO[2,3A]PHENOTHIAZINE -8,13-DIONES

4.0 Heterocyclic anthracene-9,10-dione systems

Heterocyclic analogues such as **(51 and 65)** have shown potential as highly effect agents in the treatment of cancer[1,2].

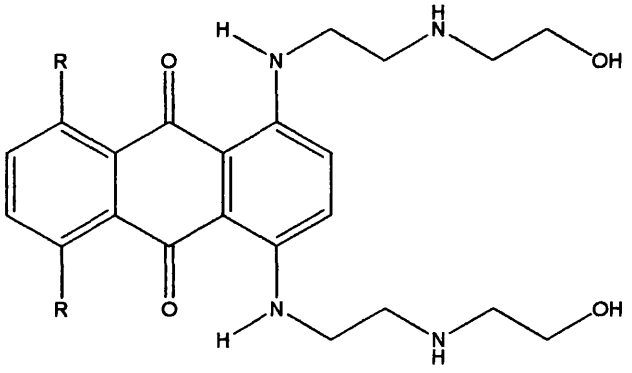


(51)
(a) X= H
(b) X= OH



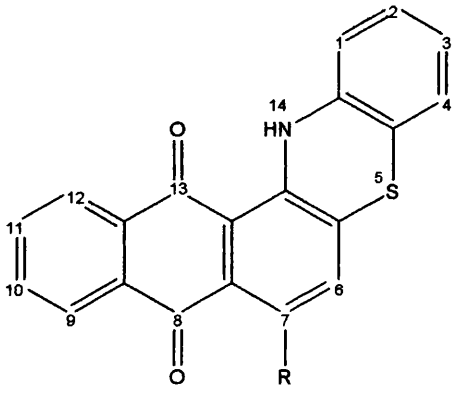
(65)

Although their activity does not exceed the parent drugs Mitoxantrone **(24a)** and Amentantrone **(24b)** (see section 1.2, p14), they have shown activity on cells which are MDR (multi drug resistant, see section 1.3.1, p33) and they have also shown a reduced cardiotoxicity.



(24)
(a) R= H Ametantrone
(b) R= OH Mitoxantrone

The study of the heterocyclic anthracene-9,10-dione nucleus is a relatively unexplored area[3], though they would be expected to retain the same spatial and planar characteristics for DNA interaction. The presence of hetero atoms introduces extra bonding or basic or reactive sites, and therefore, hetero-analogues potentially have improved activity, and possibly reduced cardiotoxicity. For example, 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione **(151b)** was developed as a blue dye for synthetic polymer fibres but its biological activity has not been reported.



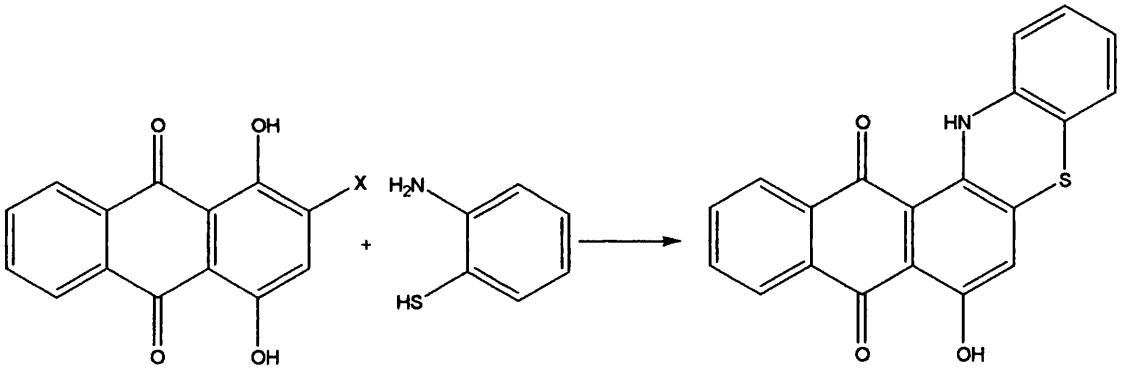
(151)
(a)R=H
(b)R=OH
(c)R=NHC$_6$H$_5$

If the appropriate aminoalkyl side chains were introduced into the 14H-naptho[2,3-a]phenothiazine-8,13-dione at position seven, it would offer the possibility of some biological effect, perhaps similar to the activity displayed by Mitoxantrone **(24b)** and Amentantrone **(24a)** (see section 1.2, p14). The presence of the nitrogen atom in the molecule might increase the anthracene-9,10-dione molecules affinity for DNA and improve the ability of the chromophore to intercalate[2], which the presence of sulfur would have significant effect on the electronic properties of the anthracene-9,10-dione

moiety and act as an additional biologically reactive centre[4] (see section 2.2). Attempts were made therefore to synthesis the parent molecule (**151**) and some of its derivatives.

4.1 Chemistry of 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-diones

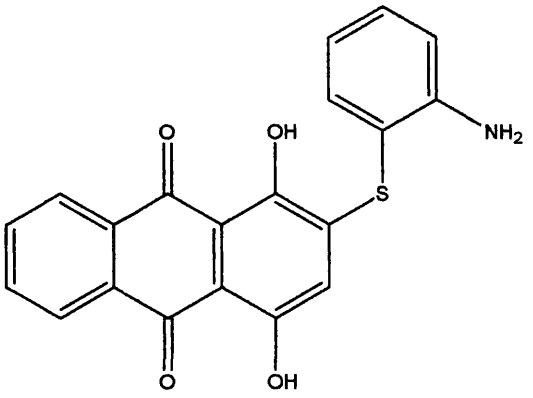
The reported synthesis of 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione (**151b**) is achieved by the condensation reaction of 2-substituted-1,4-dihydroxyanthracene-9,10-diones (**150**) with 2-aminothiophenol followed by the elimination of the 2-substituents[5].



(150) (151b)

Scheme 4-1. Synthesis of 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione (151)

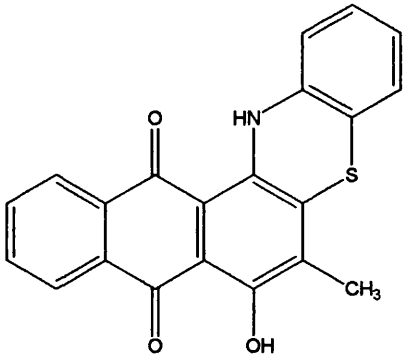
The formation of 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione proceeds via the intermediate 2-thioether (**152**).



(152)

Figure 4-1. 1,4-Dihydroxy-2-(2-aminophenyl)thioanthracene-9,10-dione intermediate

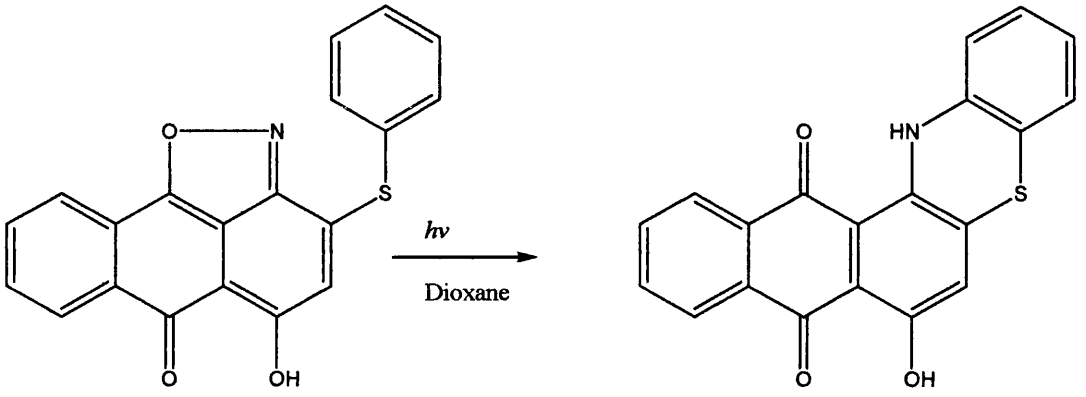
The substituents at position two (**150**) do not need to be electron attracting substituents such as halogen, as the elimination of electron donating substituents also occurs and includes methoxy, hydroxyl, and ethylamino groups. The presence of methyl substituent at position two, however leads to the formation of the 6-alkyl derivative (**153**).



(153)

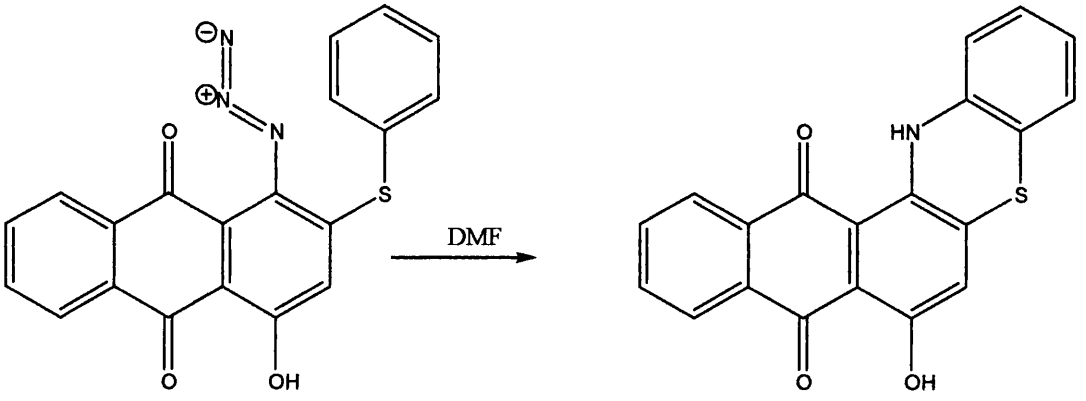
1,4-Dihydroxy-, 1-hydroxy-4-amino-, and-1-hydroxy-4-arylamino- anthracene-9,10-diones all undergo a direct thiolation and ring closure to yield 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione (**151b**).

7-Hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione (**151b**) can also be obtained from the irradiation of 6-hydroxy-3-phenylsulfanyl-anthra(1,9-cα)isoxazol-5-one (**154**) in dioxane.



(154) (151b)

The same product (**151b**) can also be obtained from the cyclisation of 1-azido-4-hydroxy-2-phenylsulfanylanthracene-9,10-dione (**155**) in DMF under reflux [6].



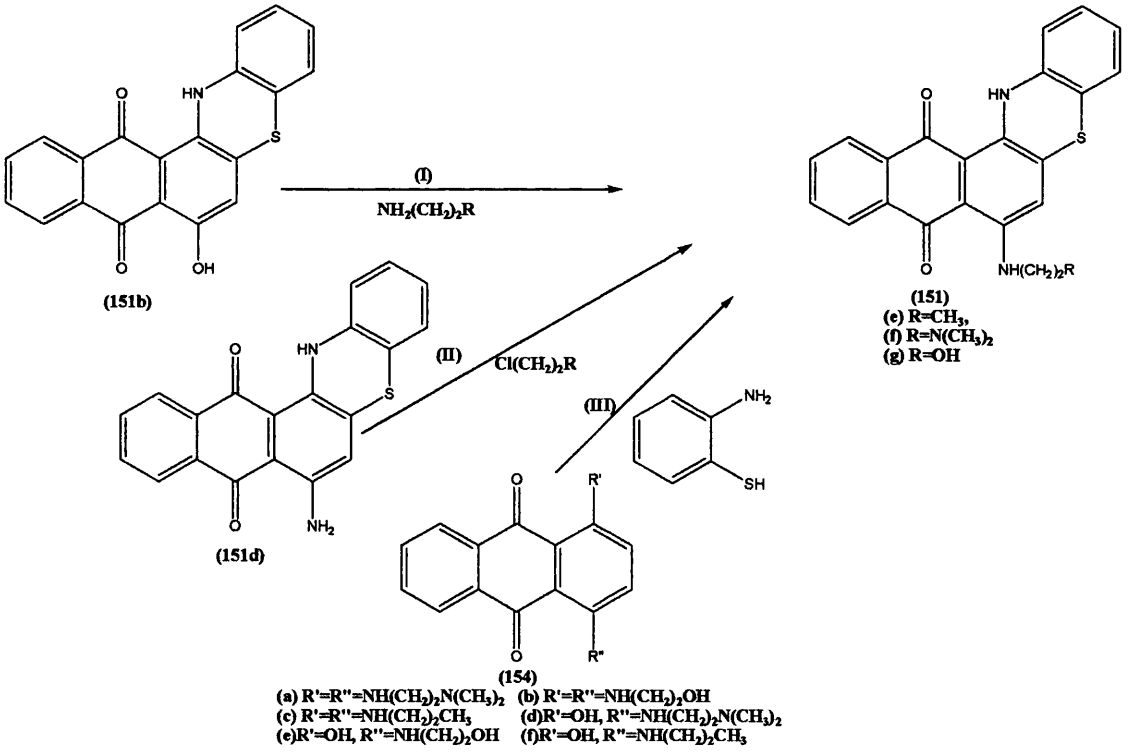
(155) (151b)

4.2 Synthetic strategy

In this work three strategies were attempted in the synthesis of the 7-(alkylamino)-14H-naptho[2,3-a]phenothiazine-8,13-diones (**151e,f,g**).

These were;

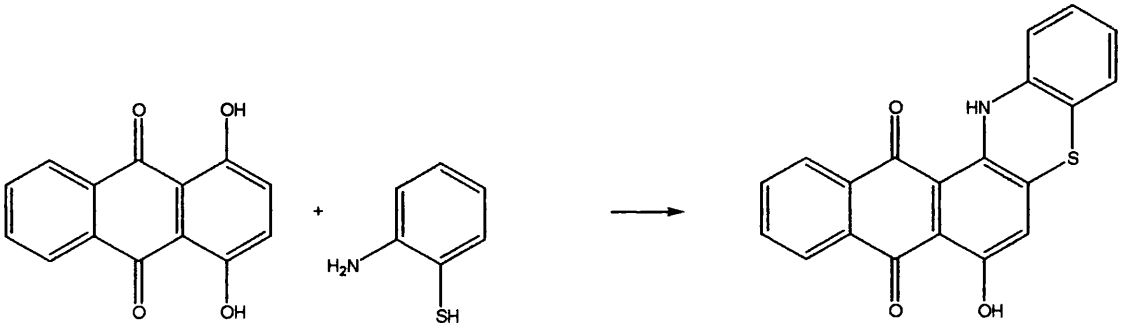
1. Replacement of the hydroxyl group in position seven of 7-(alkylamino)-14H-naptho[2,3-a]phenothiazine-8,13-dione (**151b**) with an alkyl amine.
2. Alkylation of 7-amino-14H-naptho[2,3-a]phenothiazine-8,13-dione (**151d**)
3. Direct thiolation of 1-(alkylamino)-4-hydroxyanthracene-9,10-diones (**154**) with 2-aminothiophenol.



Each will be discussed in turn.

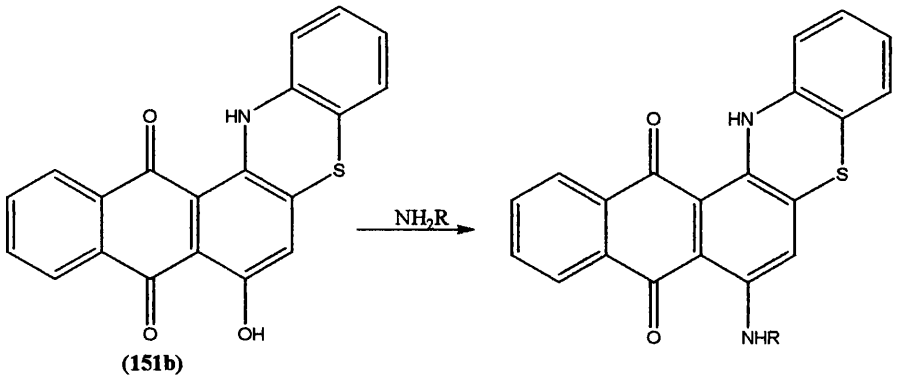
4.2.1 Synthetic strategy (I)

The synthesis of 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione was achieved in this work from the reaction 1,4-dihydroxyanthracene-9,10-dione with 2-aminothiophenol in DMF at 140-145°C (yield, 28%) or 2-methoxyethanol and boric acid under reflux (yield, 63%). It was also synthesised using 2,3-dihydro-9,10-dihydroxyanthracene-1,4-dione (the reduced or leuco form of 1,4-dihydroxyanthracene-9,10-dione) with 2-aminothiophenol in 2-methoxyethanol with boric acid leading to a greater yield (71%)



Scheme 4-2. Synthesis of 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione (151b) from 1,4-dihydroxyanthracene-9,10-dione (111)

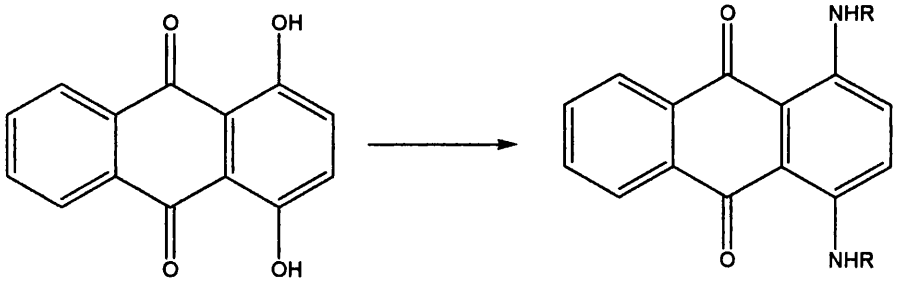
Once 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione **(151b)** was synthesised and purified, the replacement of the hydroxyl group with an alkylamine was attempted.



(151b)

Scheme 4-3. Attempted replacement of the hydroxyl group by an amino group.

This reaction is analogous to the reaction of 1,4-dihydroxyanthracene-9,10-dione **(111)** with alkylamines using a mixture of sodium carbonate with phenol in ethanol[7], to yield the corresponding 1,4-bis(aminoalkyl)anthracene-9,10-dione **(Scheme 4-4)**,



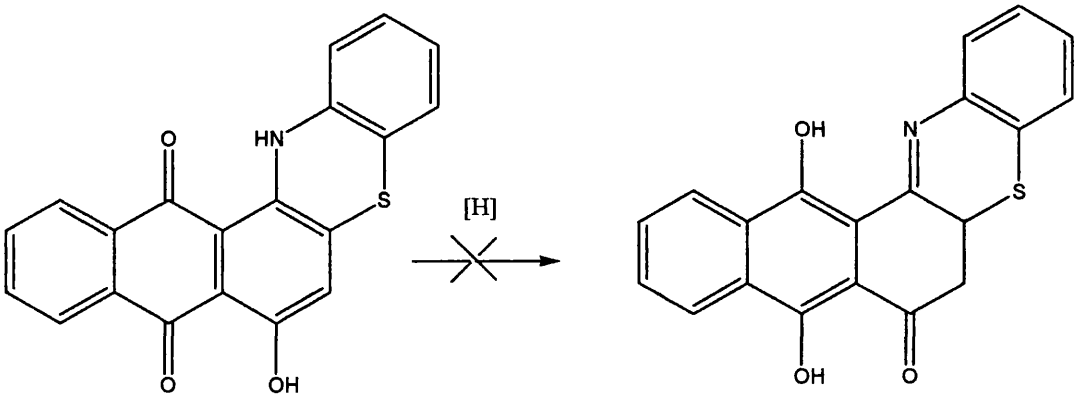
(111)

Scheme 4-4. Synthesis of 1,4-bis(aminoalkyl)anthracene-9,10-diones from 1,4-dihydroxyanthracene-9,10-dione

However unlike the 1.4-dihydroxyanthracene-9,10-dione the attempted displacement of the hydroxyl group was unsuccessful as the reaction only yielded the starting materials. The synthesis of 1,4-bis(aminoalkyl)anthracene-9,10-diones can be improved by using the reduced form of the 1,4-dihydroxyanthracene-9,10-dione (2,3-dihydro-9,10-dihydroxyanthracene-1,4-dione) **(110)** and then reacting it with the appropriate amine(

see section 1.2.1.). Reduction of 1,4-dihydroxyanthracene-9,10-dione is achieved using tin in acetic acid or aqueous sodium dithionite to yield 2,3-dihydro-9,10-dihydroxyanthracene-1,4-dione **(110)** [8,9,10].

The reduction of 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione **(151b)** was attempted using (**Scheme 4-5**) firstly sodium dithionite and then tin in acetic acid, however both of these methods were unsuccessful, and the starting material was recovered unchanged from the reaction mixture.



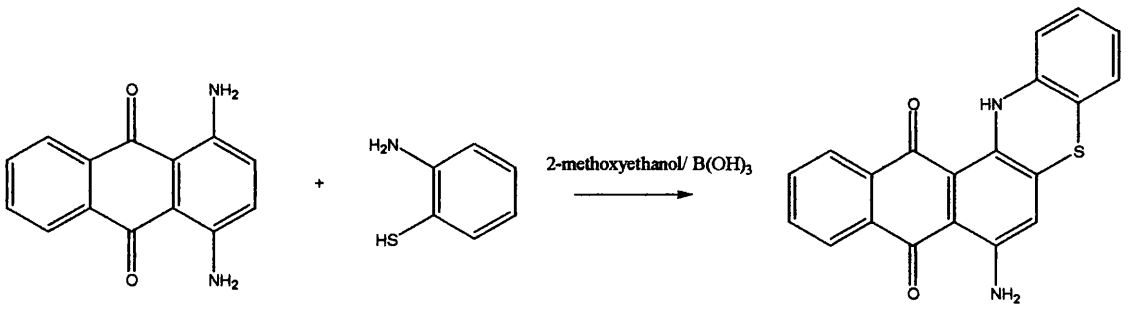
(151b)

Scheme 4-5. The attempted reduction of 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione

4.2.2 Synthetic strategy (II)

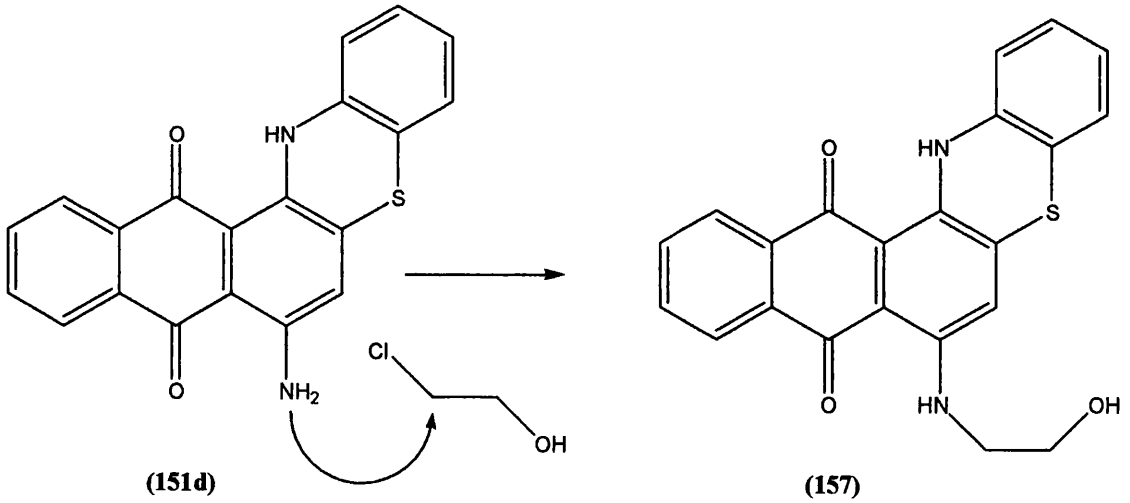
Although 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione does not react, the alternative possibility of using the accessible 1-amino-14H-naptho[2,3-a]phenothiazine-8,13-dione **(151d)** was explored.

7-Amino-14H-naptho[2,3-a]phenothiazine-8,13-dione[11] **(151d)** was synthesised in 39% yield from the reported reaction of 1,4-diaminoanthracene-9,10-dione **(148)** with 2-aminothiophenol and boric acid in 2-methoxyethanol under reflux[12]



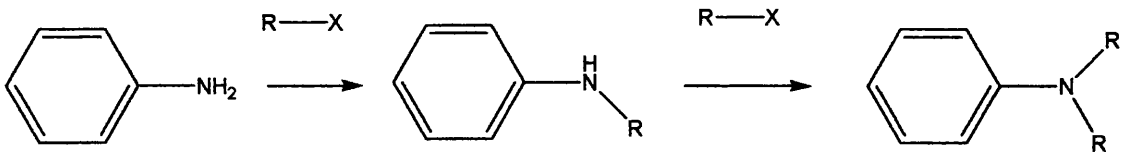
(148) **(151d)**

Once synthesised it was proposed to react 7-amino-14H-naptho[2,3-a]phenothiazine-8,13-dione **(151d)** with 2-chloroethanol in an attempt to produce 7-[2-(hydroxyethyl)amino]-14H-naptho[2,3-a]phenothiazine-8,13-dione **(157)** by displacement of the chlorine atom.



(151d) **(157)**

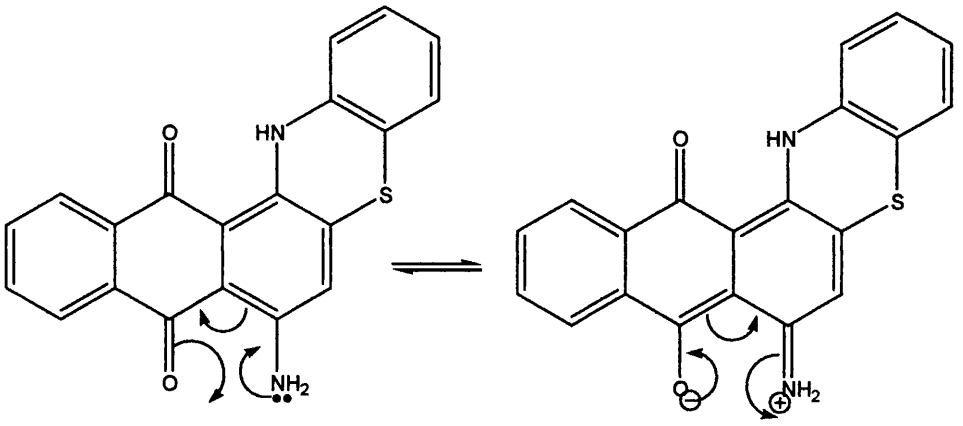
As it is well established that alkyl or aryl amines will react with alkyl halides to form secondary and tertiary amines



Scheme 4-6. Reaction of amines with alkyl halides.

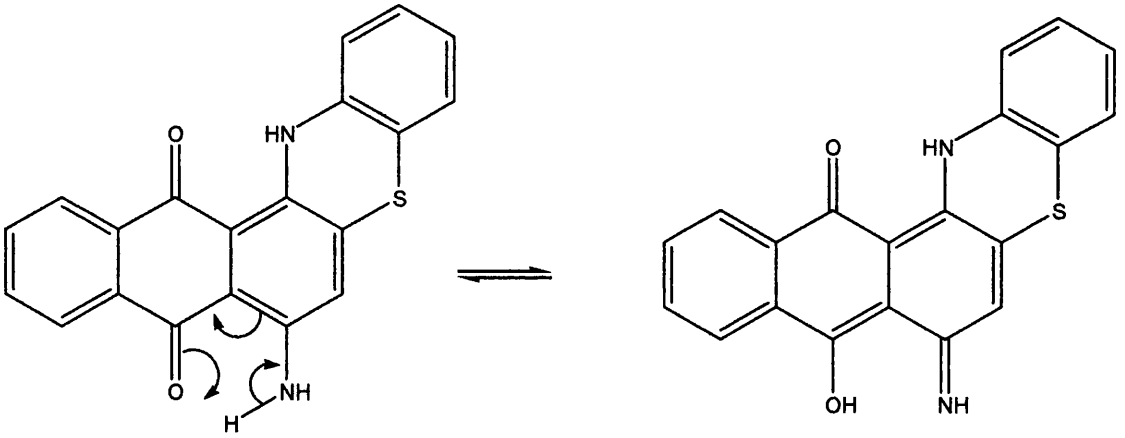
7-Amino-14H-naptho[2,3-a]phenothiazine-8,13-dione and 2-chloroethanol were refluxed in ethanol (bp 79°C), but no reaction occurred and the starting material was recovered. The same reaction was also carried out using 2-methoxyethanol (bp 125°C) as the solvent to raise the temperature of the reaction mixture but again this resulted in the recovery of the starting material. DMF (bp 153°C) was then employed as the solvent but this also proved fruitless as again only the starting material was recovered.

The failure of this reaction can be attributed to the size of the attacking nucleophile and electronic factors. Firstly the size hinders its effectiveness as a nucleophile and steric effects play a part in nucleophilic attack on the carbon containing the chlorine atom; the energy of the transition state is increased the more crowded it becomes, therefore there is a higher energy barrier to overcome before the reaction can proceed. Secondly, electronic effects reduce the nucleophilic character of the amino group by delocalisation of electron density on the anthracene-9,10-dione chromophore (Scheme 4-7).



Scheme 4-7. Delocalisation of electron density on the anthracene-9,10-dione chromophore

Thirdly, the molecule has the ability to exist in two tautomeric forms (Scheme 4-8).



Keto form

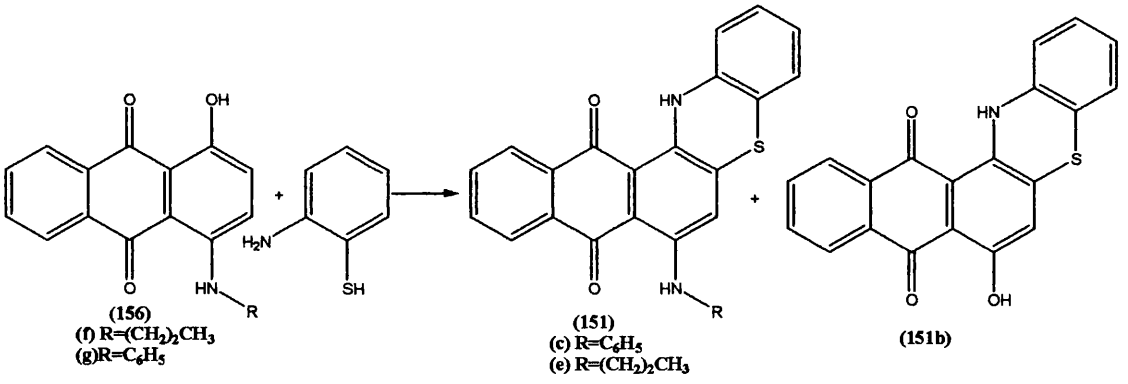
Imine form

Scheme 4-8. Tautomerisation of -amino-14H-naptho[2,3-a]phenothiazine-8,13-dione

If the molecule is in the imine form the reaction will not proceed.

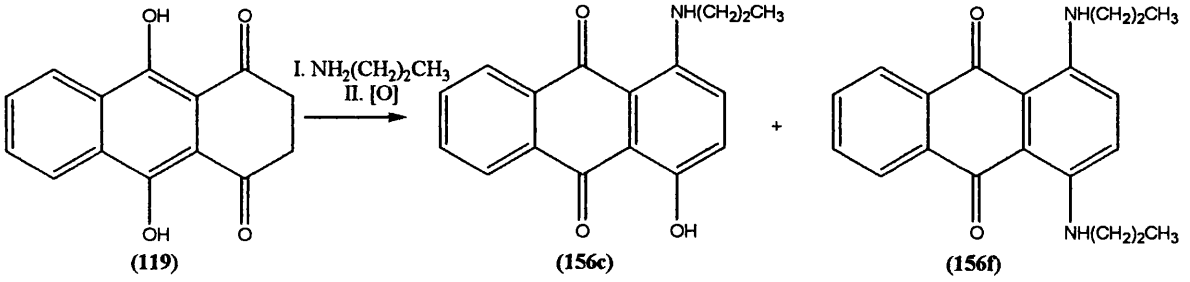
4.2.3 Synthetic strategy (IIIa)

After failing to displace the hydroxyl group of 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione **(151b)** with alkylamines and the failure to effect the nucleophilic attack on 2-chloroethanol with 7-amino-14H-naptho[2,3-a]phenothiazine-8,13-dione **(151d)**, it was decided to introduce a propylamino group onto anthracene-9,10-dione moiety to give 1-propylamino-4-hydroxyanthracene-9,10-dione **(156f)** and then react it with 2-aminothiophenol to give 7-propylamino-14H-naptho[2,3-a]phenothiazine-8,13-dione**(151e)** . A similar type of reaction is known to occur with 1-aminophenyl-4-hydroxyanthracene-9,10-dione **(156g)** and 2-aminothiophenol leading to a mixture of 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione**(151b)** and 7-aminophenyl-14H-naptho[2,3-a]phenothiazine-8,13-dione **(151c)**[13].



The synthesis of 1-(propylamino)-4-hydroxyanthracene-9,10-dione **(156f)** was achieved by reacting 2,3-dihydro-9,10-dihydroxyanthracene-1,4-dione **(119)** in ethanol at 50°C with propylamine in an equimolar or less ratio, to give a mixture of 1-(propylamino)-4-hydroxyanthracene-9,10-dione **(156c)** in 65% yield and 1,4-bis(propylamino)anthracene-9,10-dione **(156f)** in 25% yield. The desired product, 1-(propylamino)-4-

hydroxyanthracene-9,10-dione **(156c)** was then isolated by column chromatography using a eluent of 95% toluene and 5% chloroform.



(119) **(156c)** **(156f)**

Scheme 4-9. Synthesis of 1-hydroxy-4-(propylamino)anthracene-9,10-dione

The product was identified by its well resolved ^1H NMR spectrum, which showed an interesting splitting pattern in the aromatic region **(Figure 4-2)**.

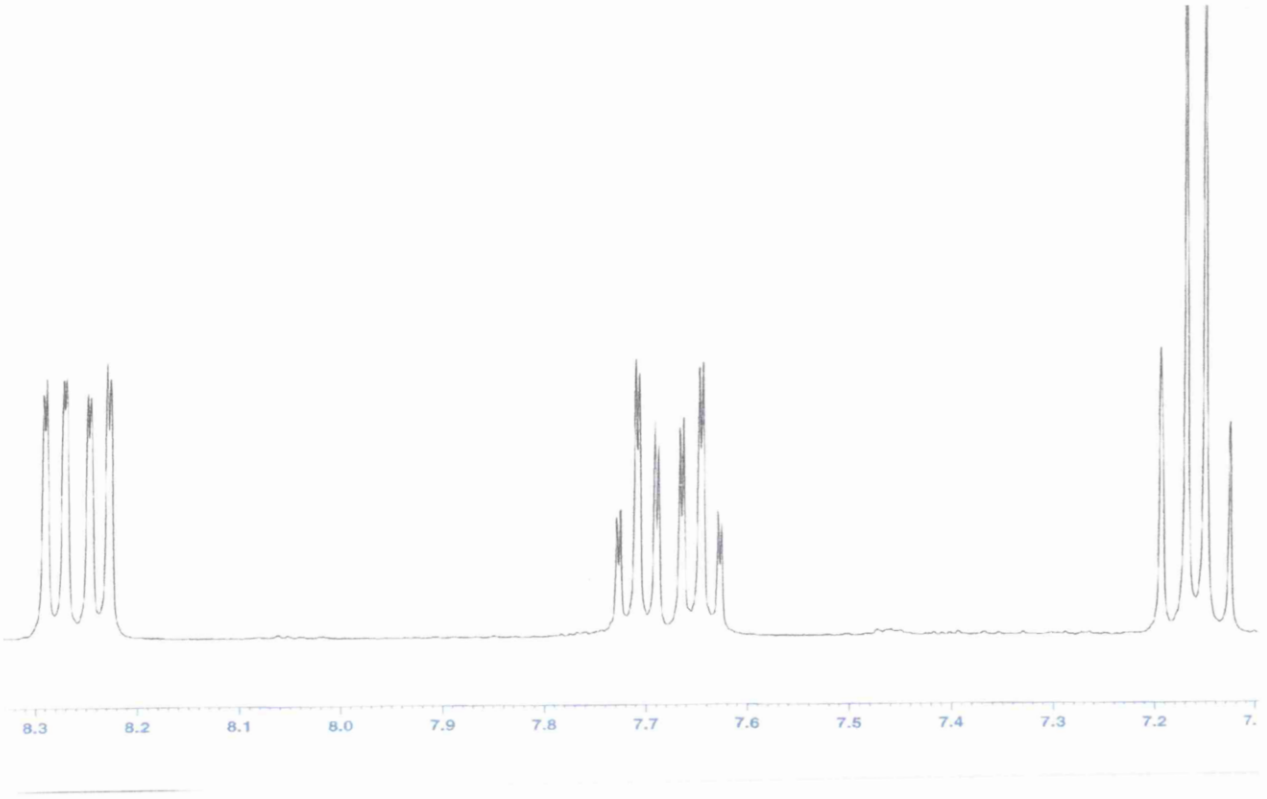
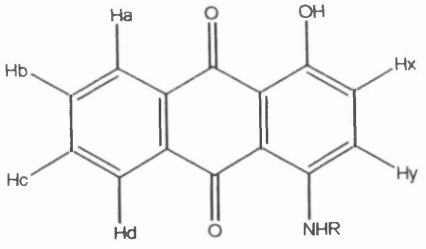


Figure 4-2. δH aromatic region for 1-hydroxy-4-(propylamino)anthracene-9,10-dione in $CDCl_3$



The assignments are shown on the next page.

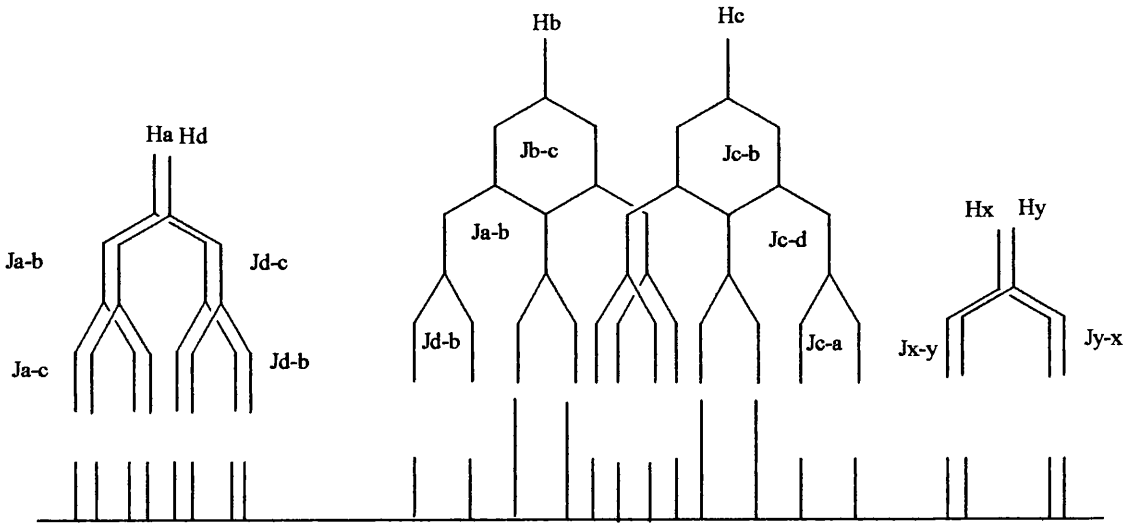
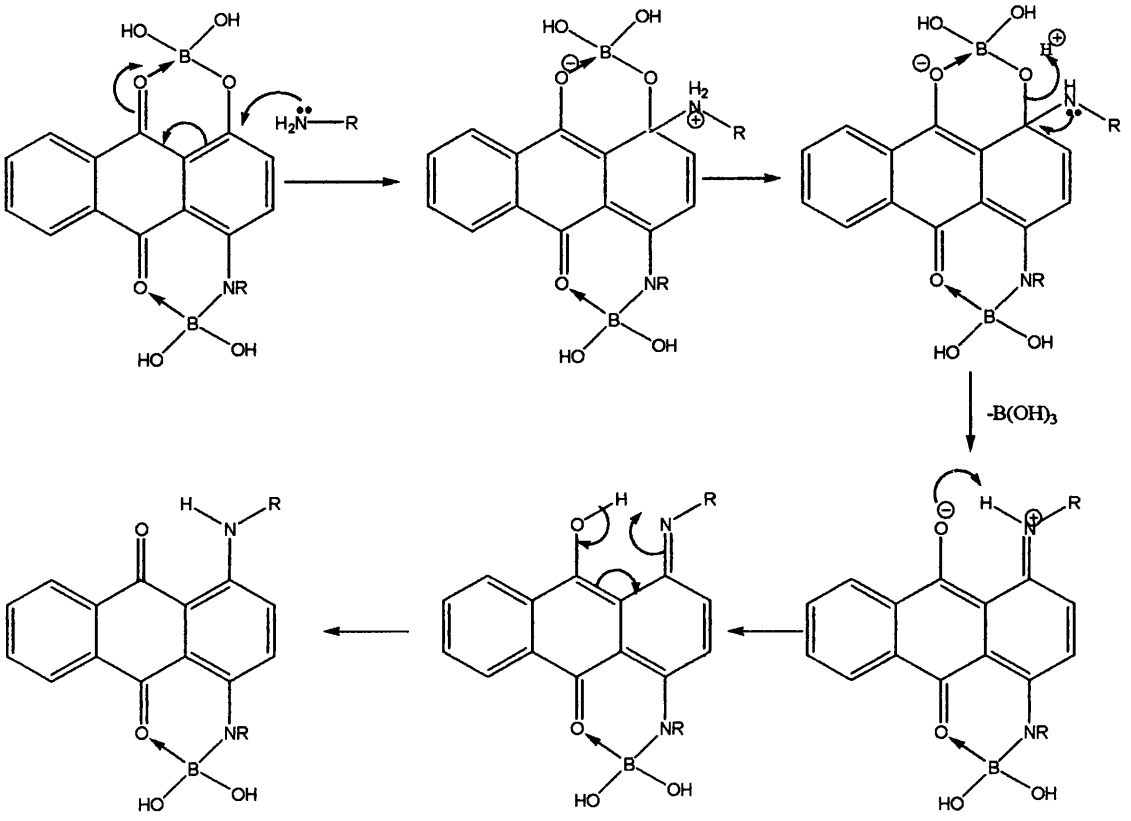


Figure 4-3. δH aromatic splitting pattern for 1-hydroxy-4-(propylamino)anthracene-9,10-dione

Although the reaction of 1-aminopropyl-4-hydroxyanthracene-9,10-dione **(156f)** with 2-aminothiophenol in DMF did not yield any product and resulted in the recovery of the starting material, the corresponding reaction of 1-aminopropyl-4-hydroxyanthracene-9,10-dione and 2-aminothiophenol **(156f)** in 2-methoxyethanol with boric acid yielded 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione and 7-aminopopyl-14H-naptho[2,3-a]phenothiazine-8,13-dione in 35% yield.

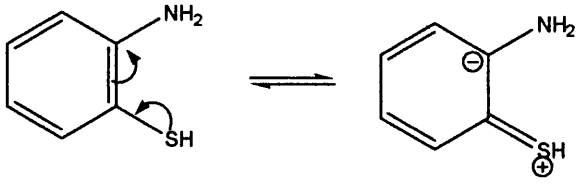
This would be consistent with the results found for the reaction of 1-aminophenyl-4-hydroxy-anthracene-9,10-dione **(156g)** to yield 7-aminophenyl-14H-naptho[2,3-a]phenothiazine-8,13-dione **(151c)** and 7-hydroxy-14H-naptho[2,3-a]phenothiazine-8,13-dione **(151b)**.

The reaction is thought to proceed via a boric ester complex (Scheme 4-10)



Scheme 4-10. Attack 2-aminothiophenol via a boric acid complex.

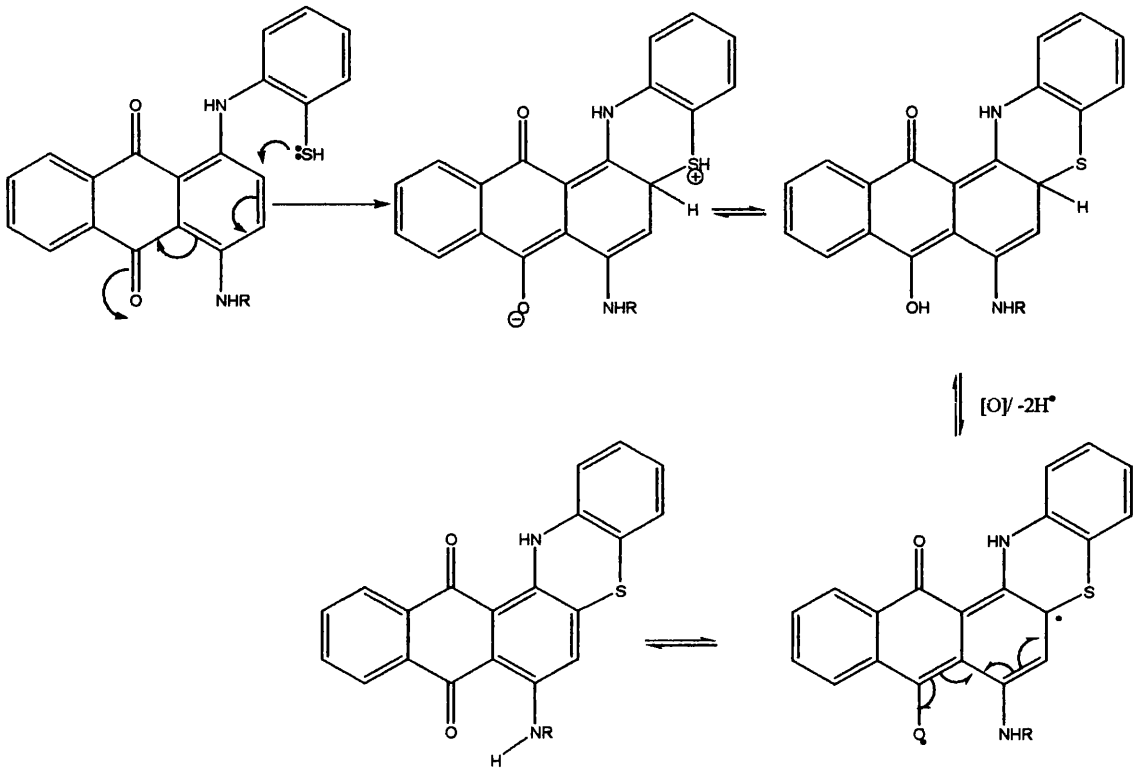
The nucleophilic character of 2-aminothiophenol is increased due to the resonance effect of the sulfur atom on the amino group (Scheme 4-11).



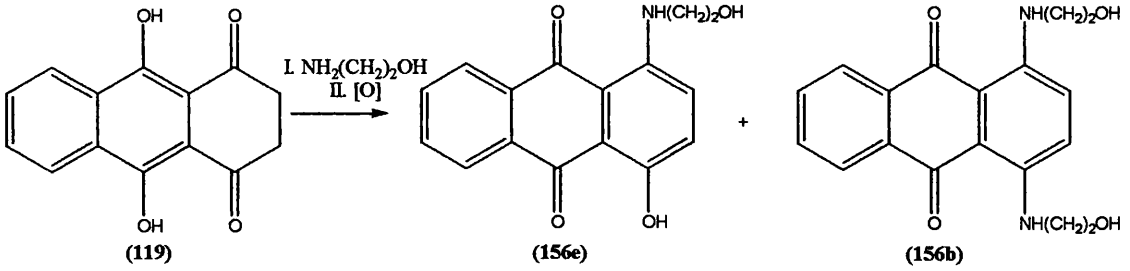
Scheme 4-11. Sulfur inductive effect.

After the nucleophilic attack on the boric ester intermediate by 2-aminothiophenol, the cyclisation of the ring can occur. Nucleophilic attack of the ring system by sulfur is

driven by the inductive effect of the carbonyl group which is *para* to the incoming nucleophile.

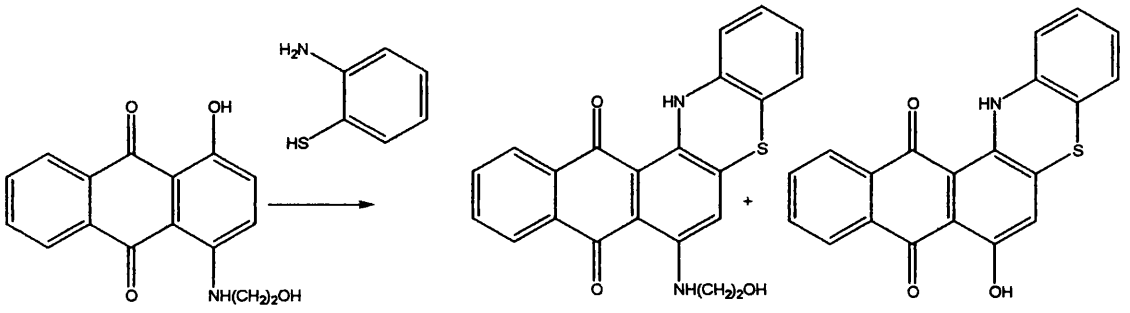


1-[2-(hydroxyethyl)amino]-4-hydroxy-anthracene-9,10-dione was also synthesised from the reaction 2,3-dihydro-9,10-dihydroxyanthracene-1,4-dione and 2-ethanolamine in ethanol to give 1-[2-(hydroxyethyl)amino]-4-hydroxy-anthracene-9,10-dione (**156e**) in 65% yield and 1,4-bis[2-(hydroxyethyl)amino]anthracene-9,10-dione in 25% yield (**156b**).



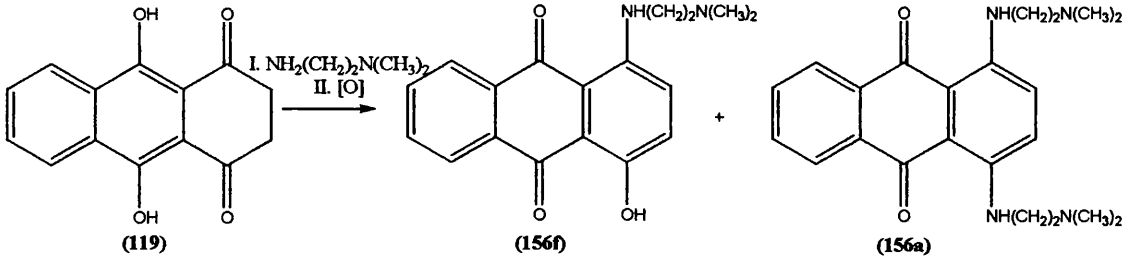
(119) (156e) (156b)

1-[2-(hydroxyethyl)amino]-4-hydroxy-anthracene-9,10-dione (**156e**) also reacted with 2-aminothiophenol using boric acid in 2-methoxyethanol to give 7-[2-(hydroxyethyl)amino]-14H-naptho[2,3a]phenothiazine-8,13-dione in 32% yield (**151g**)) and 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (**151b**) in (35%) yield.



(156c) (151g) (151b)

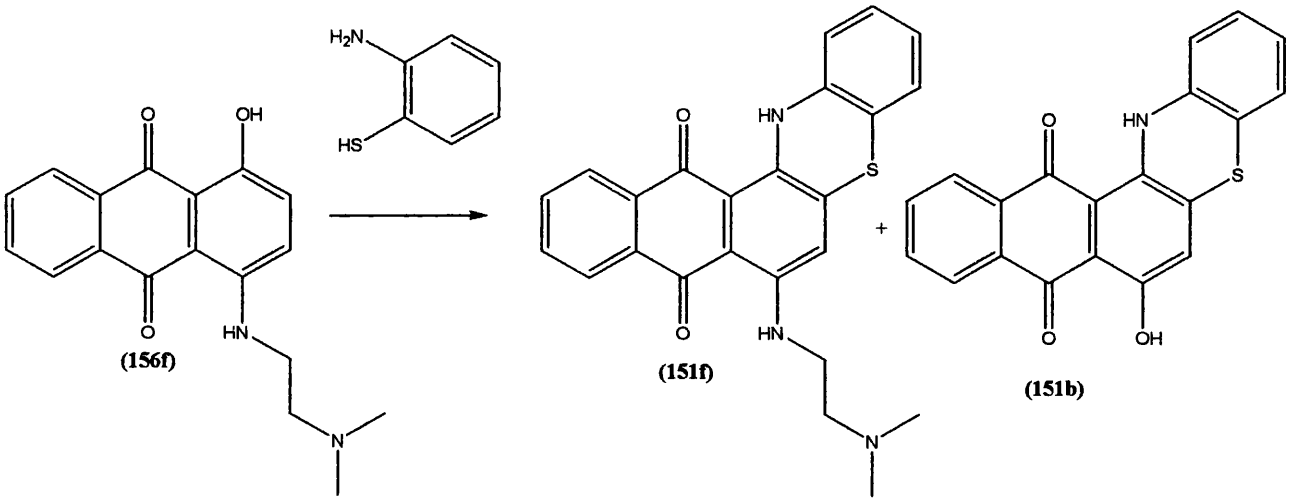
1-Hydroxy-4-{[2-(dimethylamino)ethyl]amino}anthracene-9,10-dione (**156f**) and 1,4-bis{[2-(dimethylamino)ethyl]amino}anthracene-9,10-dione (**156a**) from 2,3-dihydro-9,10-dihydroxyanthracene-1,4-dione with N,N-dimethylethylenediamine in ethanol in 55% and 25% yield respectively.



(119) (156f) (156a)

1-Hydroxy-4-{[2-(dimethylamino)ethyl]amino}anthracene-9,10-dione (**156f**) was reacted with 2-aminothiophenol and boric acid in 2-methoxyethanol to give 7-{[2-

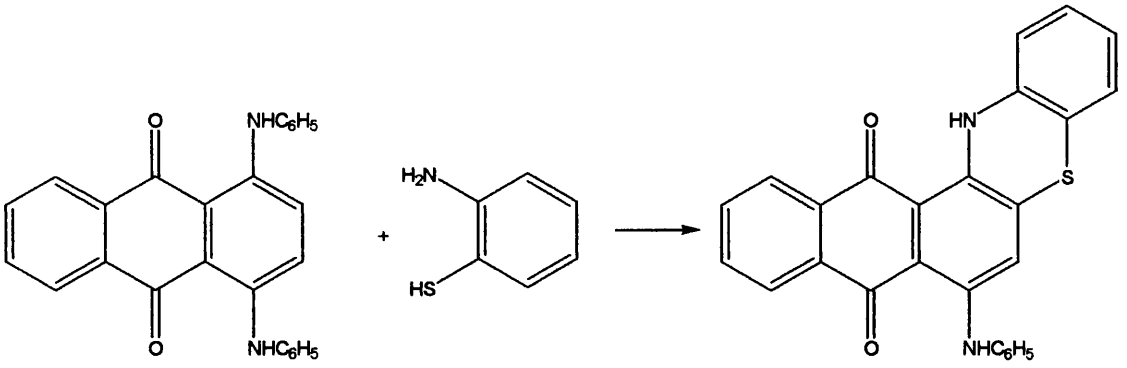
(dimethylamino)ethyl]amino}-14H-naptho[2,3a]phenothiazine-8,13-dione **(151f)** in 30% yield and 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione **(151b)** in 35% yield.



Formation of 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione **(151b)** occurs due to the attack by the 2-aminothiophenol at the carbon containing the aminoalkyl group.

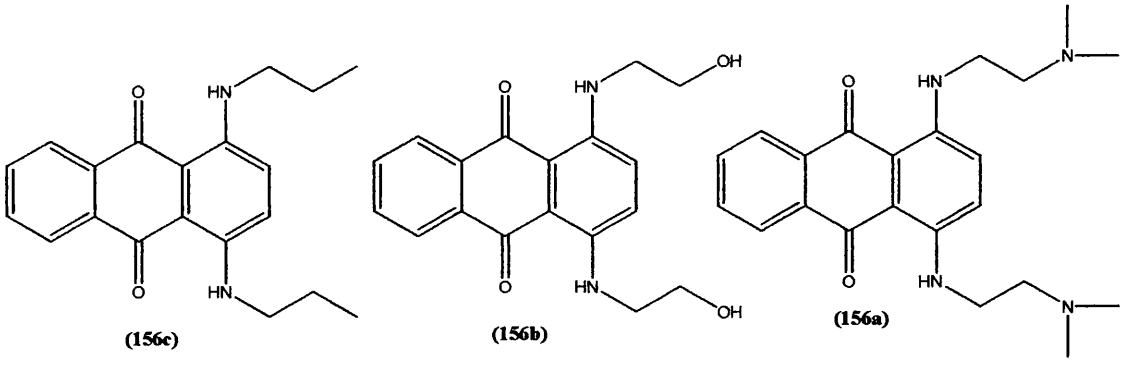
4.3 Synthetic strategy (IIIb)

1,4-Bis(aminophenyl)anthracene-9,10-dione **(157)** under goes thiolation with 2-aminothiophenol in 2-methoxyethanol with boric acid to yield 7-aminophenyl-14H-naptho[2,3-a]phenothiazine-8,13-dione[13] **(151c)**.



(157) (151c)

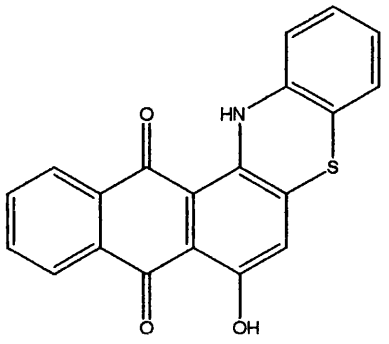
1,4-Bis(propylamino)anthracene-9,10-dione **(156c)**, 1,4-bis[2-(hydroxyethyl)amino]anthracene-9,10-dione **(156b)** and 1,4-bis{{2-(dimethylamino)ethyl]amino}anthracene-9,10-dione **(156a)**, were synthesised in an attempt to repeat the afore mentioned reaction with alkylamino substituents, however it was unsuccessful and only resulted in the recovery of the starting materials



The reaction of the alkylamino anthracene-9,10-diones with 2-aminothiophenol probably does not occur as the alkylamino substituents would push more electron density into the anthracene-9,10-dione moiety when compared to aminoaryl substituents, making the anthracene-9,10-dione moiety less susceptible to nucleophilic attack.

4.4 Experimental

4.4.1 Synthesis of 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (151b)



(151b)

1,4-Dihydroxyanthracene-9,10-dione (0.5g, 2mmol) was added to a solution of 2-aminothiophenol (2.48g, 20mmol) in DMF (15ml). The mixture was heated under reflux for 2 h, allowed to cool, and methanol was added (50ml). The resulting precipitate was filtered off and washed with warm methanol (50ml) to yield 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (0.2g, 28%), mp 257°C lit[14] 261°C, δ_H ($CDCl_3$) 6.80-7.00(5H, m) 7.75 (2H, m), 8.30 (2H, m), 12.65 (1H, *NH*, s), 14.15 (1H, *OH*, s).

1,4-Dihydroxyanthracene-9,10-dione (0.5g 2mmol) was refluxed in a solution of 2-methoxyethanol (20ml) and boric acid (0.62g 10mmol) for 30 min[13]. The solution was allowed to cool and 2-aminothiophenol was added (2.5g 20mmol), The mixture was heated under reflux for 30 min and then allowed to cool, methanol (50ml) was added to the resulting precipitate which was filtered off and washed with warm methanol (50ml), to yield 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (0.45g , 63%), mp 257°C

lit[15] 261°C, δ_H ($CDCl_3$) 6.80-7.00 (5H, m) 7.75 (2H, m), 8.30 (2H, m), 12.65 (1H, *NH*, s), 14.15 (1H, *OH*, s).

4.4.2 Attempted reduction of 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (131b)

7-Hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (1.1g, 3.3 mmol) was added to a mixture of tin (3g, 24.4mmol) and HCl (70ml) in acetic acid (50ml). The mixture was heated to 90-95°C for 24 hrs, the solution was allowed to cool and the precipitate was filtered off, the solid was washed with water and dried in vacuo to yield the starting material 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (0.8g, 72%), identification was achieved via tlc using a eluent of 5% chloroform and 95% toluene(Rf =0.61).

7-Hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (1.1g, 3.3mmol) was added to a solution of sodium carbonate (1.4g, 13.2mmol) and water (125ml). The solution was boiled and sodium dithionite (1.7g, 9.8mmol) was added, the precipitate was filtered off and washed with dilute acetic acid until alkali free, to yield the starting material 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (0.7g, 63%), identification was achieved via tlc using a eluent of 5% chloroform and 95% toluene(Rf =0.61).

4.4.3 Reaction of 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione

4.4.4 Propylamine

7-Hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (0.5g, 1.3mmol) was added to a solution of propylamine (0.77g, 13mmol) and sodium carbonate (0.5g, 4.3mmol), in

ethanol (25ml), and the mixture was heated under reflux for 24 h, allowed to cool and the precipitate was filtered off to yield the starting material 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (0.3g, 60%), identification was achieved via tlc using a eluent of 5% chloroform and 95% toluene(Rf =0.61).

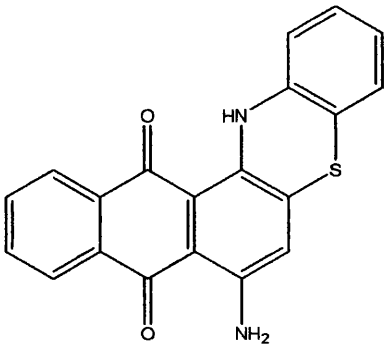
4.4.5 N,N-dimethylethylenediamine

7-Hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (0.5g, 1.3mmol) was added to a solution of N,N-dimethylethylenediamine (1.51g 13mmol) and sodium carbonate (0.5g, 4.3mmol), in ethanol (25ml), and the mixture was heated under reflux for 24 h. The solution was allowed to cool and the precipitate was filtered off to yield the starting material 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (0.2g, 40%), identification was achieved via tlc using a eluent of 5% chloroform and 95% toluene(Rf =0.61).

4.4.6 2-Aminoethanol

7-Hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (0.5g, 1.3mmol) was added to a solution of 2-aminoethanol (0.79g 13mmol) and sodium carbonate (0.5g), in ethanol (25ml) and the mixture was heated under reflux for 24 h. The solution was allowed to cool and the precipitate was filtered off to yield the starting material 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (0.3g, 60%), identification was achieved via tlc using a eluent of 5% chloroform and 95% toluene(Rf =0.61).

4.4.7 Synthesis of 7-amino-14H-naptho[2,3a]phenothiazine-8,13-dione[15] (151d)



(151d)

1,4-Diaminoanthracene-9,10-dione (0.5g, 2.1mmol) was added to a solution of 2-methoxyethanol (20ml) and boric acid (0.62g, 10mmol), and the mixture was heated under reflux for 30 min. The solution was allowed to cool, 2-aminothiophenol was added (2.5g, 20mmol), and the resulting mixture was heated under reflux for 20 min and then allowed to cool, and methanol (50ml) was added. The precipitate was filtered off and washed with warm methanol (50ml), to yield 7-amino-14H-naptho[2,3a]phenothiazine-8,13-dione (0.28g, 39%), mp 308°C, (lit[16] 310-312 °C).

4.4.8 Reaction of 7-amino-14H-naptho[2,3a]phenothiazine-8,13-dione with 2-chloroethanol

7-Amino-14H-naptho[2,3a]phenothiazine-8,13-dione (0.5g, 1.45mmol) and 2-chloroethanol (1.16g, 14.5mmol) were added to ethanol (25ml), the mixture was heated under reflux for 24 h and allowed to cool. The precipitate was collected by filtration to yield the starting material 7-amino-14H-naptho[2,3a]phenothiazine-8,13-dione (0.4g, 80%), identification was achieved via tlc using a eluent of 5% chloroform and 95% toluene(Rf =0.61).

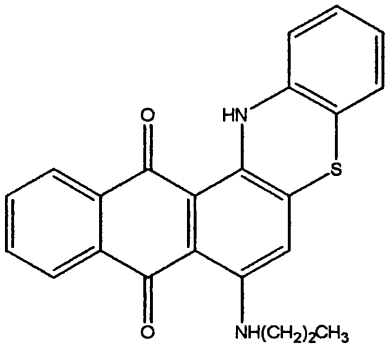
7-Amino-14H-naptho[2,3a]phenothiazine-8,13-dione (0.5g, 1.45mmol) and 2-chloroethanol (1.16g 14.5mmol) were added to 2-methoxyethanol (25ml), the mixture was heated under reflux for 24 h and allowed to cool. The precipitate was collected by filtration to yield the starting material 7-amino-14H-naptho[2,3a]phenothiazine-8,13-dione (0.4g, 80%), identification was achieved via tlc using a eluent of 5% chloroform and 95% toluene(Rf =0.61).

7-Amino-14H-naptho[2,3a]phenothiazine-8,13-dione (0.5g, 1.45mmol) and 2-chloroethanol (1.16g 14.5mmol) were added to DMF (25ml), the mixture was heated under reflux for 24 h and allowed to cool. The precipitate was collected by filtration to yield the starting material 7-amino-14H-naptho[2,3a]phenothiazine-8,13-dione (0.2g 40%) identification by TLC.

4.4.9 1-Hydroxy-4-(propylamino)anthracene-9,10-dione

2,3-Dihydro-9,10-dihydroxyanthracene-1,4-dione (1.0g, 4.2mmol) was added to a solution of propylamine (0.2g 4.2mmol) in ethanol (25ml) and heated to 50°C. The reaction mixture was allowed to cool and the precipitate was filtered off, washed with ethanol and then purified via column chromatography using an eluent of 95% toluene 5% chloroform to yield 1-hydroxy-4-(propylamino)anthracene-9,10-dione (0.50 g 45%) mp 115 °C lit 116°C[17] and 1,4-bis(propylamino)anthracene-9,10-dione (0.28g 25%) mp 176°C lit 179-180°C[8].

4.4.10 Synthesis of 7-(propylamino)14H-naptho[2,3a]phenothiazine-8,13-dione (151e)



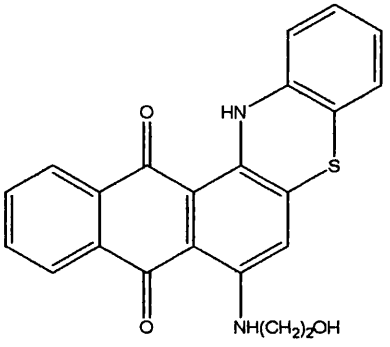
(151e)

1-Hydroxy-4-(propylamino)anthracene-9,10-dione (0.56g, 2mmol) and boric acid (0.62g, 10mmol) were refluxed in a solution of 2-methoxyethanol (20ml) for 30 min. The solution was allowed to cool and 2-aminothiophenol was added (2.5g, 20mmol), the mixture was heated under reflux for 30 min and then allowed to cool, methanol (50ml) was added and the resulting precipitate was filtered off and washed with warm methanol (50ml). The solid was purified via column chromatography using an eluent of 95% toluene 5% chloroform to yield 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione (0.24g 35%) and 7-(propylamino)14H-naptho[2,3a]phenothiazine-8,13-dione mp 250°C (0.25g 32%), mp 230 °C, δ_H 0.95 (3H, t), 1.60 (2H, m), 3.00 (2H, q), 6.35 (1H, s) 6.60 (3H m), 6.85 (1H, m), 7.50 (2H, m), 8.10 (2H, m), 10.65 (1H,*NH*, t), 12.95 (1H *OH* s). δ_c 182.93, 179.13, 147.52, 134.95, 134.58, 133.56, 132.56, 132.00, 131.55, 130.48, 126.71, 124.98, 124.77, 124.26, 121.79, 116.05, 114.77, 113.65, 108.47, 106.43, 43.51, 21.50, 10.72.

4.4.11 1-hydroxy-4-[2-(hydroxyethyl)amino]anthracene-9,10-dione

2,3-Dihydro-9,10-dihydroxy-1,4-anthracene-9,10-dione (1.0g, 4.2mmol) was added to a solution of ethanolamine (1.85g, 21mmol) in ethanol (25ml) and heated to 50°C. The reaction mixture was allowed to cool and the precipitate was filtered off. The solid was washed with ethanol and then purified via column chromatography using an eluent of 95% toluene 5% chloroform to yield 1-hydroxy-4-[2-(hydroxyethyl)amino]anthracene-9,10-dione mp 148°C (lit 150°C[18]), (0.77g, 65%) and 1,4-bis[2-(hydroxyethyl)amino]anthracene-9,10-dione (0.28g, 25%), mp 220°C (lit 242°C[8])

4.4.12 Synthesis of 7-[2-(hydroxyethyl)amino]14H-naptho[2,3a]phenothiazine-8,13-dione (151g)



(151g)

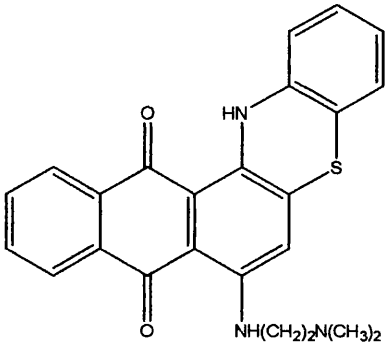
1-Hydroxy-4[2-(hydroxyethyl)amino]anthracene-9,10-dione (0.57g, 2mmol) and boric acid (0.62g, 10mmol) were refluxed in a solution of 2-methoxyethanol (20ml) for 30 min. The solution was allowed to cool and 2-aminothiophenol (2.5g, 20mmol) was added. The mixture was heated under reflux for 30 min and then allowed to cool, methanol (50ml) was added and the resulting precipitate was filtered off and washed with warm methanol (50ml). The solid was purified via column chromatography using an eluent of 95% toluene 5% chloroform to yield 7-hydroxy14H-naptho[2,3a]phenothiazine-8,13-

dione mp 255°C (0.24g 35%) and 7-[2-(hydroxyethyl)amino]14H-naptho[2,3a]phenothiazine-8,13-dione (0.25g 32%) δ_H 2.70 (2H, t), 3.45 (2H, q) 7.15 (3H, m), 7.70 (3H, m), 8.30 (3H, m), 10.35 (1H, t), 13.65 (1H, s).

4.4.13 1-hydroxy-4-{[2-(dimethylamino)ethyl]amino}anthracene-9,10-dione

2,3-Dihydro-9,10-dihydroxy-anthracene-1,4-dione (1.0g, 4.2mmol) was added to a solution of N,N-dimethylethylenediamine (1.85g 21mmol) in ethanol (25ml) and heated to 50°C. The reaction mixture was allowed to cool and the precipitate was filtered off. The solid was washed with ethanol and then purified via column chromatography with an eluent of 10% methanol and 90% chloroform, to yield 1-hydroxy-4-{[2-(dimethylamino)ethyl]amino}anthracene-9,10-dione (0.72g 55%) mp 118°C lit[19] 120°C and 1,4-bis{[2-(dimethylamino)ethyl]amino}anthracene-9,10-dione (0.4g 25%) mp 165°C lit[20] 172°C.

4.4.14 Synthesis of 7{[2-(dimethylamino)ethyl]amino}-14H-naptho[2,3a]phenothiazine-8,13-dione (151f)



(151f)

1-Hydroxy-4-{[2-(dimethylamino)ethyl]amino}anthracene-9,10-dione (0.57g 2mmol) was refluxed in a solution of 2-methoxyethanol (20ml) and boric acid (0.62g 10mmol) for 30 min. The solution was allowed to cool and 2-aminothiophenol was added (2.5g 20mmol). The mixture was heated under reflux for 30 min and then allowed to cool, to which was added methanol (50ml). The precipitate was filtered off and washed with warm methanol (50ml), the solid was purified via column chromatography with an eluent of 10% methanol and 90% chloroform to yield 7-hydroxy14H-naptho[2,3a]phenothiazine-8,13-dione (0.24g 35%) mp 210°C and 7-{[2-(dimethylamino)ethyl]amino}14H-naptho[2,3a]phenothiazine-8,13-dione (0.25g 30%) mp 220°C, δ_H 2.25 (6H, s), 2.60 (2H, t), 3.40 (2H, q) 7.15 (3H, m), 7.70 (3H, m), 8.30 (3H, m), 10.30 (1H, t), 13.65 (1H, s) δ_c 182.90, 179.18, 147.50, 134.90, 134.58, 133.56, 132.60, 132.00, 131.55, 130.48, 126.71, 124.98, 124.77, 124.26, 121.79, 116.05, 114.77, 113.65, 109.47, 106.43, 51.70, 46.80, 12.30. *m/z* (M+H) measured 416.1427 required 416.1427

4.5 References

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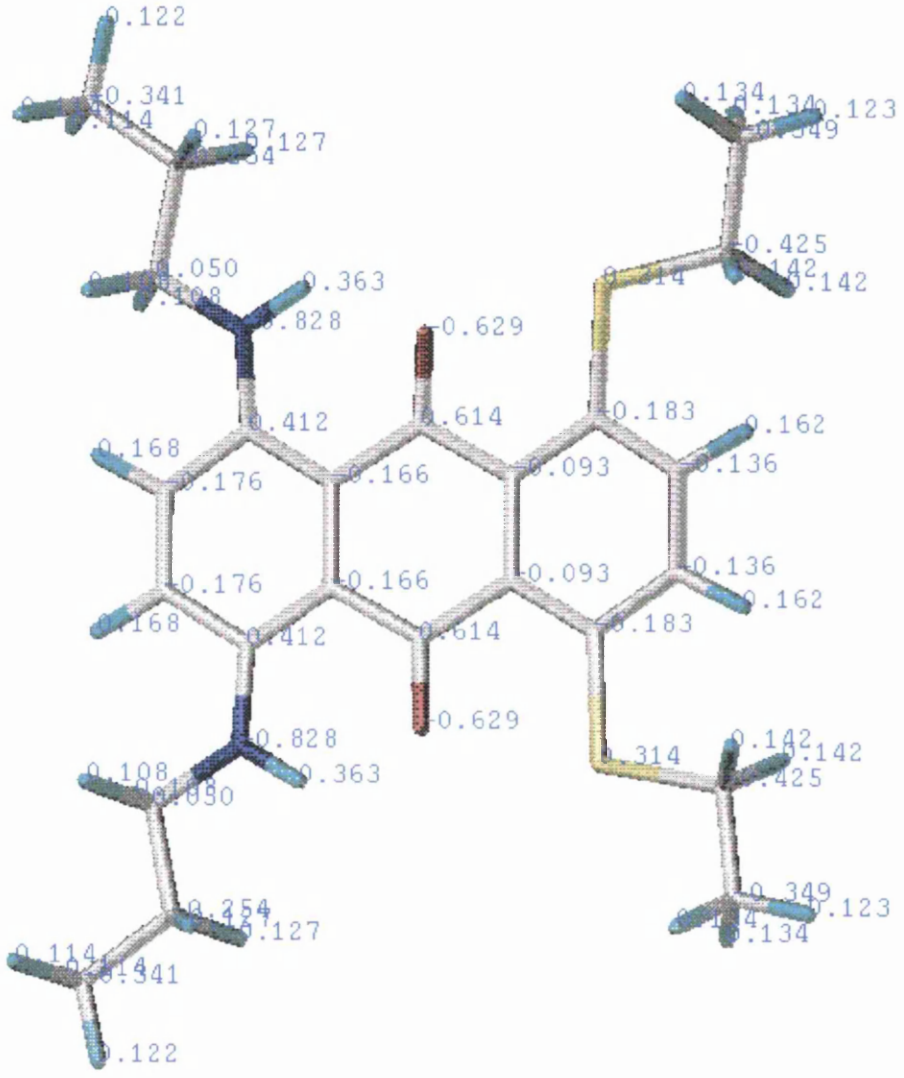
CHAPTER FIVE



COMPUTATIONAL STUDIES

5.0 Computational Studies

Computational chemistry may be defined as the application of mathematical and theoretical principles to the solution of chemical problems. Molecular modeling concentrates on predicting the behavior of individual molecules within a chemical system. The most accurate molecular models use *ab initio* (from first principles) methods, which are based upon the principles of quantum mechanics, and are generally very computer-intensive. Semi-empirical methods were therefore developed to increase the speed of computation by using approximations for large molecule systems. These methods use valence electrons only, and calculate the interactions of overlapping orbitals using preset parameters characteristic for each of the constituent elements of the system.

5.1.1 Background to Quantum Mechanics

The starting point of molecular orbital theory is the Schrödinger equation which in its simplest form can be written as (Equation 5-1)

$$H\psi=E\psi \qquad \textbf{Equation 5-1}$$

Where:

H= The Hamiltonian, which is an operator used on the wavefunction (ψ) to calculate both the kinetic and potential energies of the system of electrons and nuclei.

E= The energy of the system (also known as the eigenvalue).

ψ= The wave function (also known as an eigenfunction).

The Schrödinger equation is a partial differential eigenvalue equation; the Hamiltonian is not merely a constant to multiply by ψ and is used to obtain the energy of the system by evaluating the integral in equation 5-2

$$E=\frac{\int \psi H \psi d\tau}{\int \psi^2 d\tau} \qquad \textbf{Equation 5-2}$$

This equation is derived from equation 5-1 by multiplying by ψ and integrating over all space ($d\tau$).

The Hamiltonian operator is made up of two parts the kinetic operator - $\frac{\hbar\nabla^2}{2m}$

and the potential energy operator for an electron and a nucleus with Z protons $V= \frac{Ze^2}{4\pi\varepsilon_o r}$

Exact solutions to the Schrödinger equation can only be solved for simple systems such as the hydrogen atom or a particle in a box. If we take the most basic molecular species such as H_2^+ which contains 2 protons and an electron the Schrödinger equation is unsolvable. This is because the calculation involves knowing how the movement of each particle affects every other particle. However with the introduction of sensible approximations, calculations can be performed.

The Born-Oppenheimer approximation

As the mass of nuclei is much greater than the mass of an electron, the nuclei can be regarded as being stationery and the Born Oppenheimer approximation treats nuclei as being fixed in space. The total wavefunction can then be split into nuclear and electronic parts which can be written as:

$$\psi_{total}(\text{nuclei, electrons}) = \psi(\text{nuclei})\ \psi(\text{electrons}) \qquad \textbf{Equation 5-3}$$

The total energy equals the sum of the nuclear energy (the electrostatic repulsion between positively charged nuclei) and the electronic energy (kinetic and potential energy of the electrons moving in the electrostatic field of the nuclei, together with electron-electron repulsion):

$$E_{total} = E(\text{electrons}) + E(\text{nuclei}) \qquad \textbf{Equation 5-4}$$

The next approximation is the **variation principle** which states that the energy of the system is either greater or equal to the lowest energy of the system.

$$E = \frac{\int \psi H \psi d\tau}{\int \psi^2 d\tau} \geq E_o \qquad \textbf{Equation 5-5}$$

Molecular orbitals are constructed from a **linear combination of atomic orbitals (LCAO)** so that:

$$\psi_i = c_1\phi_1 + c_2\phi_2 + c_3\phi_3 + \ldots\ldots c_i\phi_i$$

or

$$\psi_i = \sum_{j=1}^{n} c_{ij}\phi_j \qquad \textbf{Equation 5-6}$$

where

ϕ_i = Atomic orbital located on each atom.

c_1-c_i = Mixing coefficients. These can have any value from –1 to +1 which expresses how much the particular atomic orbital contributes to the one electron wave function (molecular orbital).

i = Index number which labels the particular molecular orbital.

j = Running index of all incorporated atomic orbitals.

Combination of LCAO with equation 5-5 then yields:

$$E = \frac{\int \sum_i c_i\phi_i \, H \sum_j c_j\phi_j \delta\tau}{\int \sum_i c_i\phi_i \sum_j c_j\phi_j \delta\tau} \qquad \textbf{Equation 5-7}$$

Equation 5-7 is simplified by letting:

$$H_{ij} = \int \phi_i H \phi_j \, \delta\tau \text{ and } S_{ij} = \int \phi_i \phi_j \, \delta\tau$$

where H_{ij} are the coulomb resonance integrals and S_{ij} is the overlap integrals, thus:

$$E = \frac{\sum_i \sum_j c_i c_j H_{ij}}{\sum_i \sum_j c_i c_j S_{ij}} \qquad \textbf{Equation 5-8}$$

The equation can be expressed more generally as:

$$\sum_i c_i(H_{ij} - ES_{ij}) = 0 \qquad \textbf{Equation 5-9}$$

To solve the secular equations for the coefficients, c_i, it is necessary to know the energy E of the orbital. As for any set of simultaneous equations the secular equations have a solution if the secular determinant (the determinant of the coefficients) is zero:

$$\det\left|H_{ij} - ES_{ij}\right| = 0 \qquad \textbf{Equation 5-10}$$

Molecular orbital calculations always make use of this equation

5.1.2 Hückel Molecular Orbital

Hückel molecular orbital[1], theory is the simplest method for the calculation of molecular orbitals; it addresses the complexity of solving the Schrödinger equation by making some drastic assumptions. Firstly the conjugated π-electrons are only considered as the electrons which occupy the sigma frame are assumed to be constant and unaffected by electrons in the rest of the molecule, as they are localised between the bonded atoms. For butadiene this reduces the basis set to one atomic orbital per conjugated atom leading to an interaction matrix of only 16 terms.

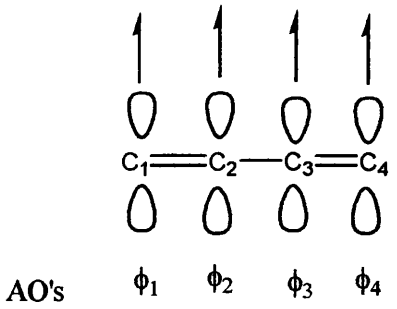


Figure 5-1. Buta-1,3-diene

The interaction matrix, permutating all pairs of AO's, is:

$$\begin{pmatrix} A_{11} & A_{12} & A_{13} & A_{14} \\ A_{21} & A_{22} & A_{23} & A_{24} \\ A_{31} & A_{32} & A_{33} & A_{34} \\ A_{41} & A_{42} & A_{43} & A_{44} \end{pmatrix}$$

A_{ij} is the interaction of orbital $_i$ with orbital $_j$

Figure 5-2. Interaction matrix for 1,3-butadiene

Since $A_{ij}=A_{ji}$ the number of interactions is reduced to 10 from 16.

if A_{ij} takes the form from equation 5-10,

$$\mathbf{A_{ij}=(H_{ij}-ES_{ij})} \qquad \textbf{Equation 5-11}$$

and,

$$\mathbf{H_{ij}=\int \phi_i H \phi_j \delta\tau \text{ and } S_{ij}=\int \phi_i\phi_j\delta\tau} \text{ (see equation 5-7)}$$

where τ is a volume element and H is the Hamiltonian operator.

The terms H_{ii} physically represent the energy of an electron in the field of its own nucleus and are known as *Columb integrals*. When considering an all carbon π-system this term can be represented by the energy of an electron in a C-$2p_z$ orbital and is regarded as constant and represented by α. The off diagonal terms, H_{ij} refer to the energy of electron in the field of nucleus and are known as the *resonance integrals*,

In the Hückel method,

For adjacent (i.e. bonded) atoms, H_{ij}= constant = β

For non adjacent atoms $H_{ij} = 0$

The final assumption is that the overlap integrals,

$$S_{ii} = 1 \text{ for the same atom}$$

$$S_{ij} = 0 \text{ for different atoms}$$

where S_{ij} is the spatial distribution or the amount of interpenetration or overlap. The interaction matrix for butadiene C-$2p_z$ can now be written as follows:

$$\begin{pmatrix} (\alpha\text{-}E) & \beta & 0 & 0 \\ \beta & (\alpha\text{-}E) & \beta & 0 \\ 0 & \beta & (\alpha\text{-}E) & \beta \\ 0 & 0 & \beta & (\alpha\text{-}E) \end{pmatrix}$$

This is then simplified by dividing through by β and setting $(\alpha\text{- }E)/\beta = x$,

$$\begin{pmatrix} x & 1 & 0 & 0 \\ 1 & x & 1 & 0 \\ 0 & 1 & x & 1 \\ 0 & 0 & 1 & x \end{pmatrix} = 0$$

Multiplying out the determinant gives a polynomial of the form $x^4 - 3x^2 + 1 = 0$, the roots of which are,

$x = -1.618, -0.618, +0.618, +1.618$ **Equation 5-12**

The relative energies are therefore

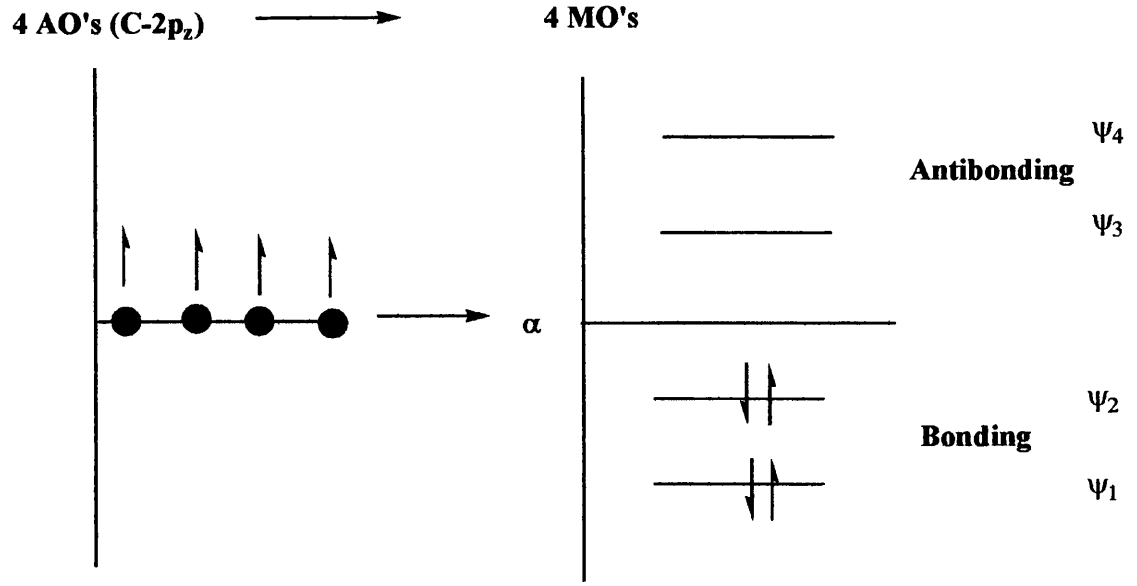
$E_1 = \alpha + 1.618\beta$ ψ_1

$E_2 = \alpha + 0.618\beta$ ψ_2

$E_3 = \alpha - 0.618\beta$ ψ_3

$E_4 = \alpha - 1.618\beta$ ψ_4

And the 4 π-electrons are accommodated in the two bonding molecular orbitals



Attention is now drawn to the electron densities at each atom. Each MO can be considered as a linear combination of its constituents AOs:

$$\psi_i = c_1\phi_1 + c_2\phi_2 + c_3\phi_3 + c_4\phi_4 \qquad \textbf{Equation 5-13}$$

In which the coefficients c_i expresses the contribution of the ith AO. Each line of the interaction matrix relates to one of a set of simultaneous equation (secular equations) containing MO coefficients c_i, as follows:

$$\begin{aligned} c_1x + c_2 + 0 + 0 &= 0 \\ c_1 + c_2x + c_3 + 0 &= 0 \\ 0 + c_2 + c_3x + c_4 &= 0 \\ 0 + 0 + c_3 + c_4x &= 0 \end{aligned}$$

together with the normalisation condition,

$$c_1^2 + c_2^2 + c_3^2 + c_4^2 = 1 \qquad \textbf{Equation 5-14}$$

These equations are solved to obtain values of the four coefficients for each orbital i.e. for each of the four values of x.

Atom	c_1	c_2	c_3	c_4
1	0.3717	0.6015	0.6015	0.3717
2	0.6015	0.3717	-0.3717	-0.6015
3	0.6015	-0.3717	-0.3717	0.6015
4	0.3717	-0.6015	0.6015	0.3717

The square of the coefficients multiplied by the orbital occupancy gives the electron density (charge) at each atom,

$$\text{Electron density at atom i, } q_i = \sum^{occi} nc_i^2$$

i.e. $q_1 = (2x \quad c_1^2) + (2x c_2^2) = (2x0.3717^2) + (2x0.6015^2) = 0.2763 + 0.7236 = 0.9999$

Therefore the electron density atom 1 will be 1 electron.

5.2 The development of Semi-empirical methods

The Hückel method from 1931 still has it uses, and is often used to find rough starting geometries or energies for more complex calculations. In 1965 Pople *et al.* [2,3] published their CNDO method which stood for "Complete Neglect of Differential Overlap". The parameters adopted were acquired from *Ab Initio* calculations and the method could yield impressive results rapidly. Other methods soon followed (See Table 5-1), most notably the MINDO/3 method devised by Dewar[4] which obtained its parameters from actual experimental data sets. It is three modern variations of this method, known by their acronyms of AM1, PM3, and MNDO which were utilized in this research.

Table 5-1: Chronological development of Semi-empirical Methods

Acronym	Method	Electrons[a]	Year[b]	Reference
HMO	Hückel Molecular Orbital.	π only	1931	1
EH	Extended Hückel Molecular Orbital.	Valence	1952	5
PPP	Pople-Pariser-Parr	π only	1957	6
CNDO/1	Complete Neglect of Differential Overlap.	Valence	1965	2
CNDO/2	Complete Neglect of Differential Overlap.	Valence	1966	7
INDO	Intermediate Neglect of Differential Overlap.	Valence	1967	8
MINDO/1/2/3	Modified INDO	Valence	1969 –1975	9,10
MNDO	Modified Neglect of Diatomic Overlap	Valence	1977	11
AM1	Austin Method 1 (modified MNDO)	Valence	1985	12
PM3	Parametric Method 3 (modified MNDO)	Valence	1989.	13

[a] electrons considered in calculation [b]Year that the method was published

5.3 Hardware and software

The molecular modelling in these studies was performed using the SYBYL[14] package whilst all the semi-empirical calculations themselves were carried out using the MOPAC 93 package[15] run on a variety of Irix networked Silicon Graphics[16] workstations. Crystal Structure data were downloaded from the Cambridge Crystallographic Database[17] and the geometries measured and properties assessed using the SYBYL package.

Due to the limited memory and disc space on the Swansea SG machines further ab initio calculations were run on the ESPRC Columbus Cluster at Rutherford Appleton laboratories. The Columbus Cluster is a midrange computing facility, consisting of a cluster of 6 Compaq

ES40 computers. Each Alphaserver ES40 machines has four 833MHz (EV68) processors with 8 GB of memory[18]. 1,4-Diamino-5,8-bis(ethylsulfanyl)anthracene-9,10-dione was run at the basis set level 4-31G** on the Columbus facility to asses the computational time required, which was two and a half hours. 1,4-diamino-5,8-bis(ethylsulfanyl)anthracene-9,10-dione was then run at the 6-31G** level, and took twenty hours to optimize. Also optimised were 1,4-bis(propylamino)-5,8-bis(ethylsulfanyl)anthracene-9,10-dione and 1,4-bis(propylamino)-5,8-bis(phenylsulfanyl)anthracene-9,10-dione at 6-31G** level.

5.4 Selection of the best semi empirical method

MOPAC contains three semi empirical methods, MNDO, AM1 and PM3 which could be used to predict properties of the anthracene-9,10-diones synthesised. To decide which method was best suited for modelling the anthracene-9,10-diones, six anthracene-9,10-dione derivatives were selected from Cambridge Structural Database[17] (CSD). These compounds were then modelled using the three computational methods and the geometries obtained were compared with geometries from the CSD.

The CSD contains crystal structure data for 224,000 small organic and organometallic molecules. This information is retrieved using QUEST, a software package designed and developed by CSD to search, display and analyse the information. The crystallographic data of the structures is assigned an R-factor which indicates how accurate the data are. Six representative anthracene-9,10-diones with good R-factors were selected for this initial investigation. These were: 1,4-dihydroxyanthracene-9,10-dione, 1,4-diaminoanthracene-9,10-dione, 1,4-bis(butylamino)anthracene-9,10-dione, 1,4-bis(isopropylamino)anthracene-9,10-dione, 1,4-bis(4-toluidinio)anthracene-9,10-dione and 1-(6-sulfanylhexylthio)anthracene-9,10-dione; each will be considered in turn.

5.4.1 1,4-Dihydroxyanthracene-9,10-dione

An examination of the computational data for 1,4-dihydroxyanthracene-9,10-dione geometries in these studies for all three methods predicts the molecule is planar (Table 5-2).

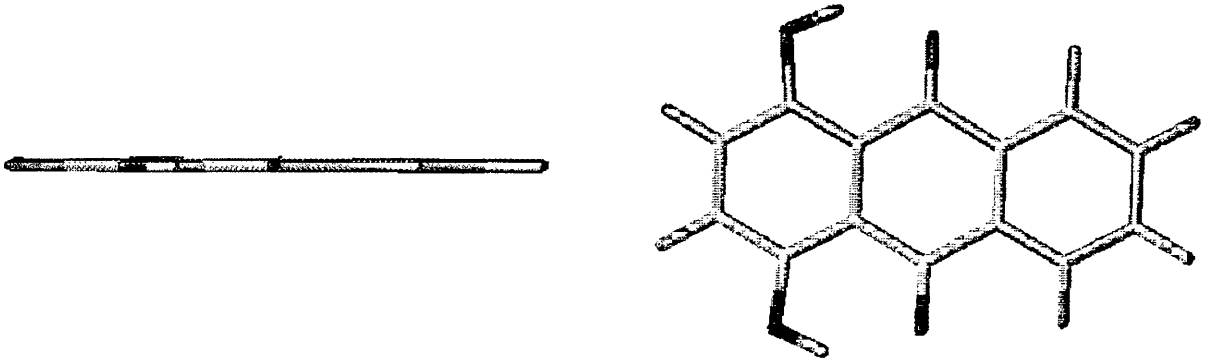
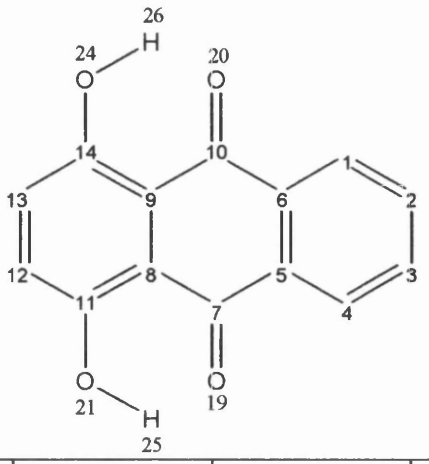


Figure 5-3. Optimised structure 1,4-dihydroxyanthracene-9,10-dione using PM3

A comparison of the bonds lengths of the actual x-ray data with the calculated data shows the differences in each of the selected methods. The most notable is the over-estimation of the hydrogen bond length O19-H25 and O20-H26 which are 1.934 and 1.932 Å for AM1 and 2.134 and 2.134 for MNDO compared to an actual of 1.757 and 1.753 Å for the x-ray data. The same overestimation of the hydrogen bond length is not seen in PM3 results which predict the bond lengths to be 1.754 and 1.794 Å (because the PM3 method was designed to reduce the over-estimation of hydrogen bonds). The other bond lengths predicted by all three methods are fairly similar to the actual ones, though overall PM3 seems to be the best method.

Table 5-2. Experimental vs. calculated geometries for 1,4-dihydroxyanthracene-9,10-dione [19]

Cambridge Database Reference Code DHXANT10	
R-Factor [f] 0.039	

Distance [a]	Cambridge	AM1	PM3	MNDO
C7-O19	1.237	1.248	1.230	1.234
C10-C20	1.236	1.245	1.230	1.234
O24-C14	1.352	1.366	1.356	1.351
C11-O21	1.352	1.366	1.356	1.351
O21-H25	0.950	0.970	0.962	0.945
O24-H26	0.950	0.970	0.962	0.945
C9-C10	1.457	1.472	1.478	1.503
C7-C8	1.457	1.472	1.478	1.503
C6-C10	1.476	1.475	1.485	1.501
C5-C7	1.471	1.475	1.485	1.501
O19-H25	1.757	1.934	1.794	2.134
H26-O20	1.753	1.931	1.794	2.134
Angle [b]				
C14-O24-H26	109.0	111.2	108.7	117.7
C11-O21-C25	109.0	111.5	108.7	117.7
C8-C7-O19	120.8	121.9	120.3	120.8
C9-C10-O20	121.0	121.9	120.2	120.8
C9-C14-O24	123.2	126.9	124.5	128.2
C8-C11-O21	123.6	126.9	124.7	128.2
Torsion [b]				
H26-O24-C14-C9	-2.9	-0.8	-0.2	0.0
H25-O21-C11-C8	-1.5	0.0	0.0	0.0
C9-C8-C7-O19	-179.6	-179.6	-179.6	180.0
C8-C9-C10-O20	-178.8	-179.8	179.9	180.0
O24-C14-C9-C8	179.6	-179.9	180.0	180.0
O21-C11-C8-C9	179.1	180.0	-179.9	180.0
Charge [c]				
O19&O20		-0.446	-0.446	-0.446
O21&O24		-0.081	-0.081	-0.081
H25&H26		0.303	0.303	0.262
Heat of formation [d]		-95.59	-108.01	-98.71
Dipole [e]		1.793	2.268	1.581

[a] Bond lengths in angstroms. [b] Bond angles in degrees. [c] MOPAC charges [d] Heat of formation in kcal mol^{-1}. [e] Dipole moment in Debyes. [f] R-factor is a measure of the agreement between the postulated structures relative to the diffractometer data collected. [g].

5.4.2 1,4-Diaminoanthracene-9,10-dione

The modelling of 1,4-diaminoanthracene-9,10-dione by all three methods did not predict an accurate structure as, all three computational methods gave a non-planar structure, contrary to the x-ray data, which predicts a planar molecule (Table 5-3). The results show that the anthracene-9,10-dione moiety has been bent around the carbonyl atoms C10 and C7, with the oxygen atoms of the carbonyl group forced out of the plane of the molecule and perpendicular to the two aromatic rings of the anthracene-9,10-dione. This distortion arises because of a non-planar amino group which is bent out of the plane of the ring. The attached hydrogen atoms are out of plane also but the hydrogen bond distance (H28-O19) is preferentially retained which is why the oxygen atoms are pushed out of plane causing the molecule to buckle. The extent of this buckling can be seen from the angle C14-C10-C1, which are 177.3° for the CSD structure and 152.1° for the structure predicted by AM1.

Figure 5-4. Predicted structure of 1,4-diaminoanthracene-9,10-dione by AM1

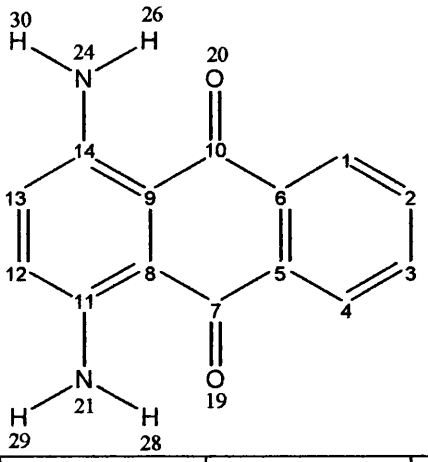
The distortion of the planar anthracene-9,10-dione moiety is prevented if the starting structure is totally planar. The structure used to carry out these calculations was taken from CSD. The initial torsion angles for the protons, show that they are slightly out of the molecules plane (C8-C11-N21-H28, 2.7° and C9-C14-N24-H26 -2.7°) but if the calculations are repeated using a completely planar starting molecule, constructed in Sybyl, the problem is resolved as all three methods give a planar amino group (Table-5-4).

Figure 5-4. Structure of 1,4-diaminoanthracene-9,10-dione predicted by PM3 using the Sybyl starting structure

However, the planar conformer is higher in energy than the original structure, with values for the AM1 method of 2.8 kcal mol^{-1}, 6.9 kcal mol^{-1} for the PM3 method and 18.4 kcal mol^{-1} for the MNDO method.

Again a closer examination reveals that the PM3 method is more accurate at predicting the hydrogen bond length H28-O19 and H26-O20 with values of 1.837 Å, 2.102 Å for the AM1 method, and 2.169 Å for the MNDO method. Other important bond lengths include O20-C10 and O19-C7 with values 1.254 Å for , N24-C14 and N21-C11 with values of, 1.254 Å, 1.348 Å and 1.348 Å which are all more accurately predict by the AM1 method which gave values of 1.247 Å, 1.247 Å, 1.391 Å and 1.391 Å respectively (Table 5-4).

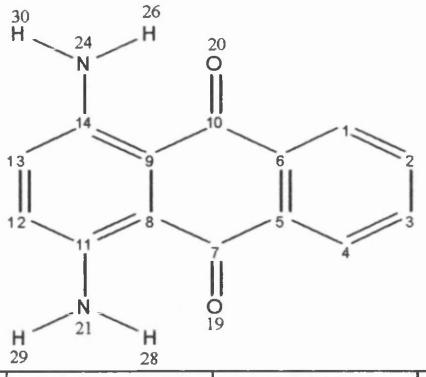
Table 5-3 Experimental vs. calculated geometries for 1,4-Diaminoanthracene-9,10-dione[20] using the CSD starting structure

Cambridge Database Reference Code GICXOF	
R-Factor [f] 0.039	

Torsion [a]	**Cambridge**	**AM1[g]**	**PM3[g]**	**MNDO[h]**
O20-C10-C9-C8	-176.0	-152.3	-158.6	152.2
O19-C7-C8-C9	176.0	152.2	158.6	152.2
C8-C11-N21-H28	2.7	22.1	2.40	-22.1
C9-C14-N24-H26	-2.7	22.1	-21.4	-22.1
C9-C8-C11-N21	-179.6	173.7	174.8	173.7
C8-C9-C14-N24	179.6	-173.8	-174.8	-173.7
Distance [b]				
O20-C10	1.254	1.241	1.224	1.241
O19-C7	1.254	1.241	1.224	1.241
N24-C14	1.348	1.391	1.423	1.391
N21-C11	1.348	1.391	1.423	1.391
H28-O19	1.929	2.104	1.892	2.104
H26-O20	1.929	2.103	1.892	2.104
C9-C10	1.440	1.479	1.487	1.479
C8-C7	1.440	1.479	1.487	1.479
C10-C6	1.479	1.479	1.487	1.479
C7-C5	1.479	1.479	1.487	1.479
Angle [a]				
C14-C10-C1	177.3	152.1	158.3	152.1
C11-C7-C4	177.3	152.1	158.3	152.1
O20-C10-C9	119.4	116.6	111.5	120.5
O19-C7-C8	119.4	116.6	111.5	120.5
C9-C10-C6	122.7	123.6	121.7	116.0
C8-C7-C5	122.7	123.6	121.7	116.0
C11-N21-H28	118.9	115.9	117.1	117.0
C14-N24-H26	118.9	115.9	117.1	117.0
Charges [c]				
O19&O20		-0.337	-0.343	-0.313
N21&N24		-0.050	-0.364	-0.338
H26&H26		0.120	0.183	0.223
Heat of formation [d]		-9.85	-14.82	-9.85
Dipole [e]		1.649	0.424	1.649

[a] Bond angles in degrees. [b] Bond lengths in angstroms. [c] MOPAC charge [d] Heat of formation in kcal mol^{-1}. [e] Dipole moment in Debyes. [f] R-factor is a measure of the agreement between the postulated structures relative to the diffractometer data collected. Calculations carried out using keywords XYZ, Geo-ok and EF. [j]

Table 5-4. Experimental vs. calculated geometries for 1,4-Diaminoanthracene-9,10-dione[20] using the planar Sybyl starting structure

Cambridge Database Reference Code GICXOF	
R-Factor [f] 0.039	

Torsion [a]	**Cambridge**	**AM1**	**PM3**	**MNDO**
O20-C10-C9-C8	-176.0	-179.9	-180.0	180.0
O19-C7-C8-C9	176.0	-180.0	-180.0	180.0
C8-C11-N21-H28	2.7	0.0	0.0	0.0
C9-C14-N24-H26	-2.7	0.0	0.0	0.0
C9-C8-C11-N21	-179.6	180.0	180.0	180.0
C8-C9-C14-N24	179.6	180.0	180.0	180.0
Distance [b]				
O20-C10	1.254	1.247	1.231	1.234
O19-C7	1.254	1.247	1.231	1.234
N24-C14	1.348	1.372	1.391	1.384
N21-C11	1.348	1.372	1.391	1.384
N21-O20	2.617	2.690	2.584	2.761
N24-O19	2.617	2.690	2.584	2.761
H28-O19	1.929	2.102	1.837	2.169
H26-O20	1.929	2.102	1.837	2.169
C9-C10	1.440	1.473	1.477	1.501
C8-C7	1.440	1.473	1.477	1.501
C10-C6	1.479	1.478	1.488	1.504
C7-C5	1.479	1.478	1.488	1.504
Angle [a]				
C14-C10-C1	177.3	178.1	177.1	177.7
C11-C7-C4	177.3	178.1	177.1	177.7
O20-C10-C9	119.4	124.0	121.6	121.8
O19-C7-C8	119.4	124.0	121.6	121.8
C9-C10-C6	122.7	119.1	118.9	120.5
C8-C7-C5	122.7	119.1	118.9	120.5
C11-N21-H28	118.9	119.7	118.9	125.7
C14-N24-H26	118.9	119.7	118.9	125.7
Charges [c]				
O19&O20		-0.343	-0.337	-0.343
N21&N24		-0.390	-0.050	-0.364
H26&H26		0.260	0.120	0.183
Heat of formation [d]		-7.02	-7.87	8.56
Dipole [e]		2.378	1.580	1.996

[a] Bond angles in degrees. [b] Bond lengths in angstroms. [c] MOPAC charge [d] Heat of formation in kcal mol^{-1}. [e] Dipole moment in Debyes. [f] R-factor is a measure of the agreement between the postulated structures relative to the diffractometer data collected. Calculations carried out using keywords XYZ, Geo-ok and EF.

5.4.3 1,4-bis(butylamino)anthracene-9,10-dione

The same problem of the nitrogen atom being forced out of plane with the molecule bent around the carbonyl atoms also occurred when 1,4-bis(butylamino)anthracene-9,10-dione was calculated.

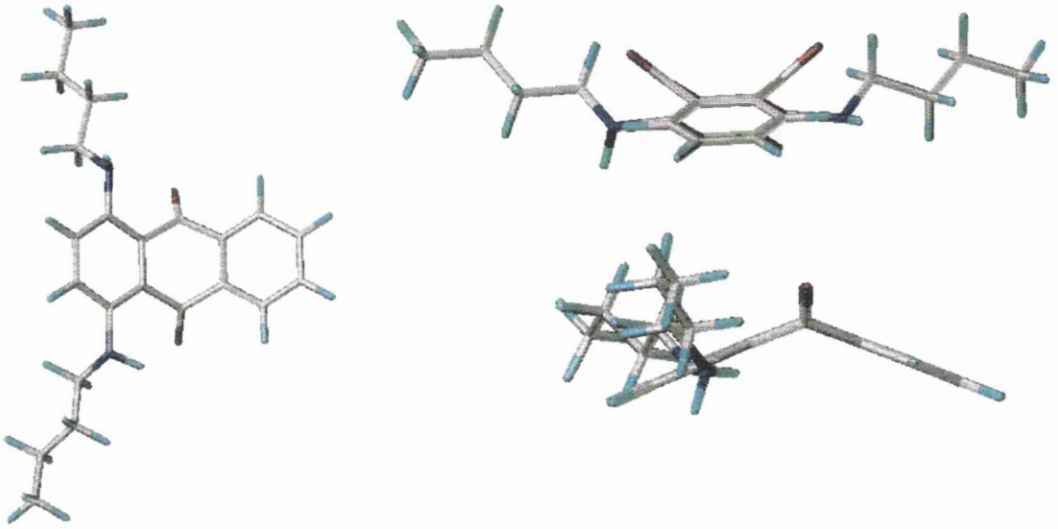


Figure 5-5. Structure of 1,4-bis(butylamino)anthracene-9,10-dione predicted by MNDO using the Sybyl starting structure

The experimental angles C17-C5-C7 and C14-C12-C10 of the molecule are 176.3° and 176.0° (Table 5-5), but with AM1 they are predicted to be 148.7° and 153.4°, PM3 gives values of 156.4° and 159.4° and MNDO, creates the greatest distortion, with values of 136.1° and 137.0°. Again the calculations were carried out using the CSD starting structure (Table 5-5), which is also not completely planar, as shown by the torsion angles H28-N4-C14-C18 and H27-N3-C17-C13 which have values of 1.2° and 9.6°.

The calculation were run again using a structure that was built in the Sybyl program (Table 5-6) but this was to no avail as the structure was again predicted to be non-planar and the nitrogen atom ended up in a tetrahedral conformation as opposed to trigonal planar arrangement in the starting structure. The tetrahedral conformation of nitrogen preferred by the AM1, PM3, and MNDO methods means the hydrogen atoms are pushed out of plane with

respect to the anthracene-9,10-dione nucleus. In order to maintain the hydrogen bond lengths the carbonyl oxygen atoms are pulled out of the plane of the molecule, causing the molecule to buckle as the rings bend away in the opposite direction.

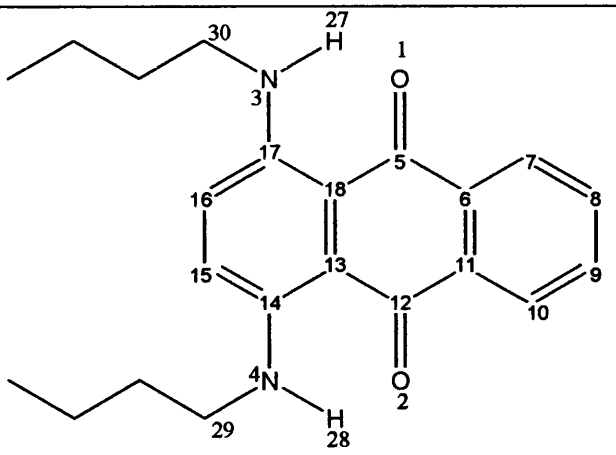
In order to prevent the molecule from buckling the ring, the carbons of the polycyclic system and the oxygen atoms were fixed in the Z-plane. This prevented the molecule from buckling at the carbonyl groups and therefore kept the molecule flat and planar but it did not prevent the nitrogen atom ending up in a tetrahedral conformation when using the PM3 method. It was necessary therefore to fix the nitrogen atoms also in the aromatic plane. Table 5-7 shows the energy differences produced in moving from an unconstrained calculation with a tetrahedral nitrogen conformation to the constrained case.

For AM1 there is a difference of 3.2 kcal mol^{-1} between the planar and non-planar conformation, while the energy differences for MNDO is 18.7 kcal mol^{-1}.

An examination of the corresponding data for PM3 shows the situation is complicated further, as there are three different energy levels. The total energy of the molecule is -49.1 kcal mol^{-1} if the nitrogen is in a tetrahedral conformation and the molecule is buckled. If the ring system is constrained the molecule remains flat but the nitrogen becomes distorted into a tetrahedral conformation, and the total energy of the molecule decreases to -48.1 kcal mol^{-1}, a difference of 1.1 kcal mol^{-1}. However if the nitrogen atom is also constrained the molecule remains planar but the penalty paid for this is a decrease in the total energy to -42.1 kcal mol^{-1}. From this it can be deduced that the constrained planar nitrogen is 5.9 kcal mol^{-1} higher in energy that a tetrahedral unconstrained nitrogen and that the planar constrained molecule is 1.06 kcal mol^{-1} higher in energy than the buckled unconstrained molecule.

The calculated data for the constrained molecule shows that the hydrogen bond distance is again overestimated by AM1 and more so by MNDO (Table 5-6) with the PM3 method being the most accurate. AM1 is also more accurate at predicting the bond lengths O1-C5, N4-C14 and N3-C17 with values of 1.240 Å, 1.387 Å and 1.376 Å compared to 1.254 Å, 1.347 Å and 1.359 Å respectively (Table 5-6) in CSD.

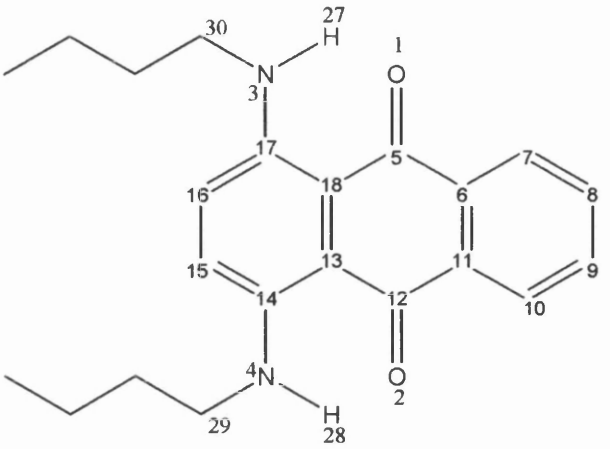
Table 5-5. Experimental vs. calculated geometries for 1,4-bis(butylamino)anthracene-9,10-dione[21] using the unconstrained AM1, PM3 and MNDO methods

Cambridge Database Reference Code CAMJOP	
R-Factor [f] 0.050	

Torsion [a]	Cambridge	AM1	PM3	MNDO
O1-C5-C18-C13	177.7	147.3	163.6	133.7
O2-C12-C13-C18	-178.9	157.1	-156.1	-136.9
N3-C17-C18-C13	179.5	-173.3	-170.2	175
N4-C14-C13-C18	-179.3	171.8	-175.5	-174.2
C30-N3-C17-C16	1.1	13.7	12.6	-48.5
C29-N4-C14-C15	-7.1	2.1	16.8	98.7
C17-C18-C5-C6	176.5	-147.6	160.6	136.1
C14-C13-C12-C11	-177.1	154.6	-156.9	-137.6
Distance [b]				
O1-H27	1.781	2.099	1.881	3.91
O2-H28	1.899	2.045	1.852	2.65
O1-C5	1.254	1.240	1.224	1.22
O2-C12	1.255	1.242	1.226	1.23
N3-C17	1.359	1.318	1.430	1.44
N4-C14	1.347	1.389	1.425	1.43
C18-C5	1.437	1.480	1.487	1.51
C13-C12	1.449	1.477	1.485	1.51
C12-C11	1.469	1.478	1.488	1.51
C5-C6	1.480	1.481	1.486	1.51
Angle [a]				
C17-N3-H27	110.2	115.1	111.2	108.4
C14-N4-H28	110.4	116.8	112.1	111.6
C18-C5-O1	123.1	123.7	121.8	124.1
C13-C12-O2	122.4	124.2	122.0	123.4
N3-H27-O1	142.7	129.8	135.7	25.89
N4-H28-O2	142.7	128.9	134.9	110.5
C17-C5-C7	176.3	148.7	156.4	136.1
C14-C12-C10	176.0	153.4	159.4	137.0
Charges [c]				
O1&O2		-0.309	-0.344	-0.342
N3&N4		-0.304	-0.001	-0.385
H27&H28		0.238	0.107	0.206
Heat of formation [d]		-40.91	-49.15	-31.97
Dipole [e]		3.077	1.905	2.38

[a] Bond angles in degrees. [b] Bond lengths in angstroms. [c] MOPAC charge [d] Heat of formation in kcal mol^{-1}. [e] Dipole moment in Debyes. [f] R-factor is a measure of the agreement between the postulated structures relative to the diffractometer data collected. Calculations carried out using keywords XYZ, Geo-ok and EF. [j]

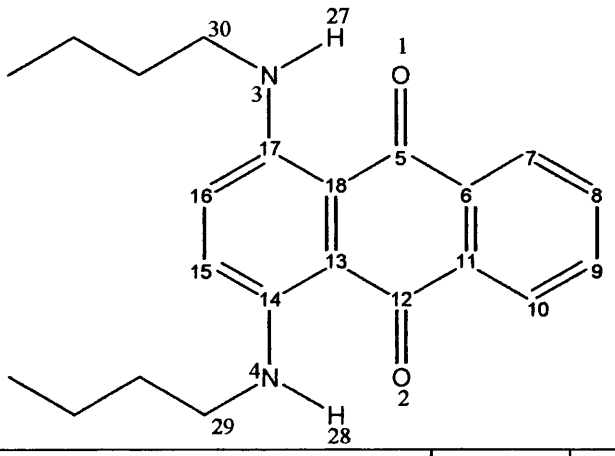
Table 5-6. Experimental vs. calculated geometries for 1,4-bis(butylamino)anthracene-9,10-dione[21] using the constrained AM1, PM3 and MNDO methods

Cambridge Database Reference Code CAMJOP	
R-Factor [f] 0.050	

Torsion [a]	Cambridge	AM1 [g]	PM3 [g]	MNDO [h]
H27-N3-C17-C18	9.6	180.0	180.0	180.0
H28-N4-C14-C13	-1.2	-180.0	-180.0	180.0
O1-C5-C18-C13	177.7	180.0	180.0	180.0
O2-C12-C13-C18	-178.9	-180.0	-180.0	180.0
N3-C17-C18-C13	179.5	180.0	180.0	180.0
N4-C14-C13-C18	-179.3	180.0	180.0	180.0
C30-N3-C17-C16	1.1	0.0	0.0	0.0
C29-N4-C14-C15	-7.1	0.1	0.1	0.0
C17-C18-C5-C6	176.5	180.0	180.0	180.0
C14-C13-C12-C11	-177.1	-180.0	-180.0	180.0
Distance [b]				
N3H27	0.805	0.999	1.000	1.012
N4H28	0.930	1.000	1.000	1.007
O1-H27	1.781	1.949	1.815	2.042
O2-H28	1.899	1.956	1.805	2.038
O1-C5	1.254	1.240	1.222	1.234
O2-C12	1.255	1.220	1.244	1.234
N3-C17	1.359	1.376	1.409	1.396
N4-C14	1.347	1.387	1.391	1.396
N3-O1	2.583	2.674	2.582	2.745
N4-02	2.586	2.681	2.579	2.747
C18-C5	1.437	1.474	1.494	1.505
C13-C12	1.449	1.486	1.470	1.505
C12-C11	1.469	1.475	1.466	1.503
C5-C6	1.480	1.476	1.477	1.504
Angle [a]				
C17-N3-H27	110.2	118.7	117.8	119.2
C14-N4-H28	110.4	119.4	118.3	119.1
C18-C5-O1	123.1	124.7	122.0	122.1
C13-C12-O2	122.4	124.7	121.7	122.1
N3-H27-O1	142.7	128.4	131.3	125.9
N4-H28-O2	142.7	127.9	130.2	126.1
C17-C5-C7	176.3	178.2	177.4	136.6
C14-C12-C10	176.0	178.0	177.5	137.0
Heat of formation [d]		-37.76	-42.15	-13.31
Dipole [e]		2.383	1.547	1.796

[a] Bond angles in degrees. [b] Bond lengths in angstroms. [c] MOPAC charge [d] Heat of formation in kcal mol^{-1}. [e] Dipole moment in Debyes. [f] R-factor is a measure of the agreement between the postulated structure relative to the diffractometer data collected Molecule constrained in the Z plane at atoms 3,4,13,14,16,17,27,28,29 & 30. [h] Molecule constrained in the Z plane at atoms ,3,4,5,12,13,14,16,17,27,28,29 & 30. Calculations carried out using keywords XYZ, Geo-ok and EF.

Table 5-7. Summary of computational data for 1,4-bis(butylamino)anthracene-9,10-dione[21]



Method	Atoms constrained in the Z plane[a]	N-H Bond	Structure	Nitrogen Conformation	Total Energy
PM3	No	Out of plane	Twisted	Tetrahedral	-49.15
PM3	O1,O2,C5,C6,C7,C8,C9,C10,C11,C12,C13,C14, C15,C16,C17,C18	Out of plane	Planar	Tetrahedral	-48.09
PM3	H27,N3,C30,C17,C18,C15,C14,N4,H28,C29	In plane	Planar	Planar	-42.15
PM3	H27,N3,C30,H28,N4,C29	In plane	Planar	Planar	-42,15
PM3	H27,N3,H28,N4	In plane	Planar	Planar	-42,15
PM3	N3,N4	In plane	Planar	Planar	-42,15
AM1	No	Out of plane	Twisted	Tetrahedral	-40.91
AM1	C5,C6,C7,C8,C9,C10,C11,C12,C13,C14,C15,C16,C17,C18	In plane	Planar	Planar	-37.74
AM1	H27,N3,C30,C17,C18,C15,C14,N4,H28,C29	In plane	Planar	Planar	-37.74
AM1	H27,N3,C30,H28,N4,C29	In plane	Planar	Planar	-37.74
AM1	H27,N3,H28,N4	In plane	Planar	Planar	-37.74
AM1	N3,N4	In plane	Planar	Planar	-37.76
MNDO	No	Out of plane	Twisted	Tetrahedral	-31.97
MNDO	O1,O2,C5,C6,C7,C8,C9,C10,C11,C12,C13,C14,C15,C16,C 17,C18	In plane	Planar	Planar	-13.31
MNDO	H27,N3,C30,C17,C18,C15,C14,N4,H28,C29	In plane	Planar	Planar	-13.31
MNDO	H27,N3,H28,N4	In plane	Planar	Planar	-13.31
MNDO	N3,N4	In plane	Planar	Planar	-13.31

[a]The input file for MOPAC is modified by freezing the position of the atoms in the Z plane for a particular atom

5.4.4 Other 1,4-Bis(alkylamino)anthracene-9,10-dione[22]

A similar distortion of the molecule is also found when 1,4-bis(isopropylamino)anthracene-9,10-dione is calculated by using the three methods (Table 5-8).

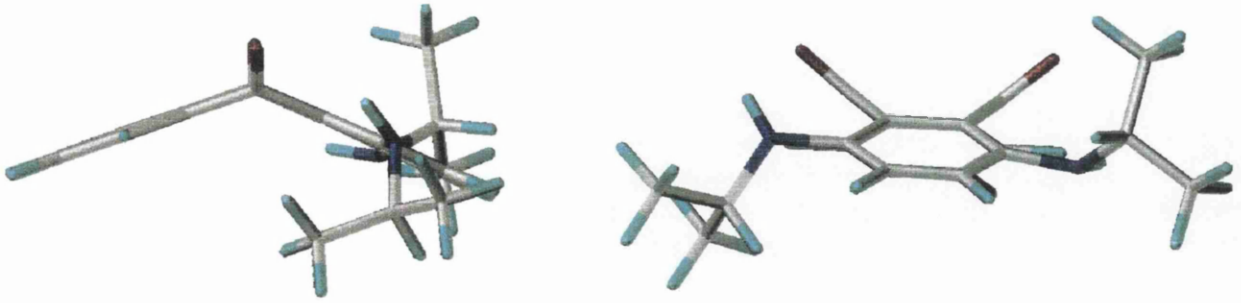


Figure 5-7. 1,4-Bis(isopropylamino)anthracene-9,10-dione structure predicted by MNDO

The buckling of the molecule is again prevented by constraining the heavy atoms of the polycyclic system and the exocyclic nitrogen's so they lie in the same plane (Table 5-9). . Again, this results in a difference of total energy for the constrained and unconstrained molecule, for the AM1 method this is 3.1 kcal mol^{-1}, for the PM3 method this is 8.5 kcal mol^{-1} and for the MNDO method this is 20.7 kcal mol^{-1}(see Table 5-10). A similar result was obtained for the simplest aminoalkyl-anthracene-9,10-dione, 1,4-bis(methylamino)anthracene-9,10-dione. This molecule was also predicted to be non planar and resulted in the buckling of the molecule and shows that the longer alkyl chain is not responsible for the non-planar structure.

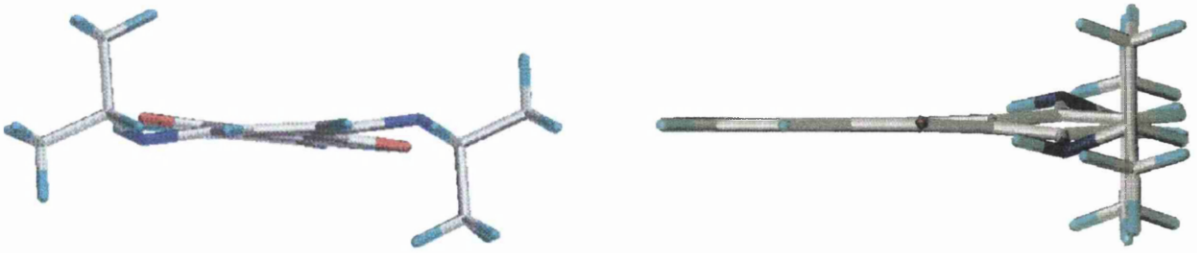
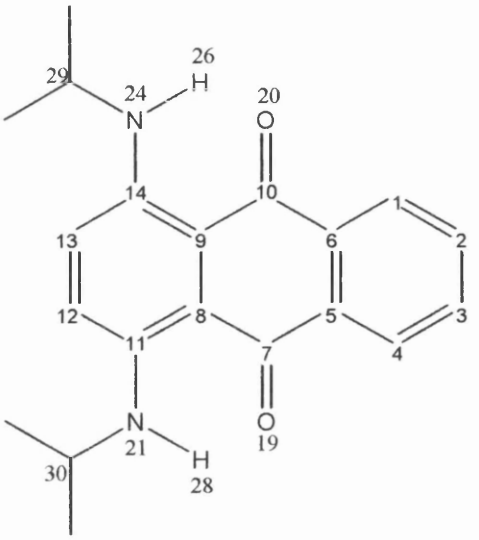


Figure 5-8. 1,4-Bis(isopropylamino)anthracene-9,10-dione modelled by PM3 with the heavy atoms of the polycyclic system constrained in the z-plane

Examination of the data obtained when the molecule is constrained in the z-plane again (Table 5-9) shows that MNDO and AM1 methods again overestimate the length of hydrogen bond, with the PM3 method being the most accurate. However, the bond length for O20-C10, and N24-C14 are more accurately predicted by the AM1 method (Table 5-9) when compared to the CSD structure.

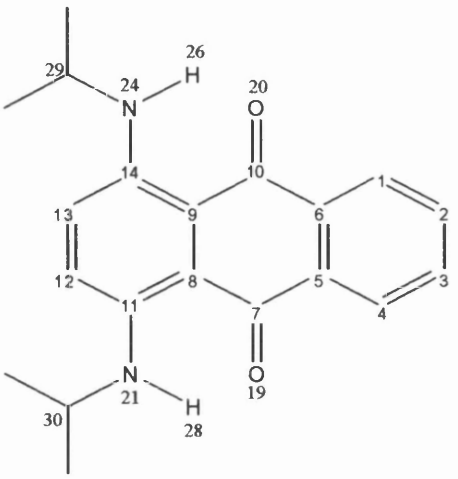
Table 5-8. Experimental vs. calculated geometries for 1,4-bis(isopropylamino)anthracene-9,10-dione[22] using the unconstrained AM1, PM3 and MNDO methods

Cambridge Database Reference Code BOCMUB	
R-Factor [f] 0.056	

Torsion [a]	Cambridge	AM1	PM3	MNDO
C13-C14-N24-C29	14.8	-160.3	34.7	-136.4
C11-C12-N21-C30	1.8	-160.7	40.4	134.1
O20-C10-C9-C8	178.9	-164.3	-155.8	171.5
O19-C7-C8-C9	177.9	-164.3	162.9	175.7
N21-C11-C8-C9	-178.4	-13.4	-169.9	-24
N24-C14-C9-C8	179.3	-13.3	-175.5	-8
H26-N24-C14-C9	11.7	40.9	-19.8	37.7
H28-N21-C11-C8	-0.4	41.3	-11.2	22.4
Distance [b]				
O20H26	1.776	1.962	1.883	2.456
O19H28	1.747	1.962	1.854	2.58
N24-C14	1.362	1.382	1.430	1.425
N21-C11	1.351	1.382	1.425	1.427
C7-O19	1.256	1.247	1.225	1.235
C10-O20	1.260	1.247	1.224	1.225
C10-O9	1.461	1.477	1.487	1.511
C7-C8	1.464	1.477	1.485	1.51
C6-C10	1.480	1.478	1.457	1.506
C5-C7	1.496	1.478	1.488	1.505
H26-N24	1.080	0.999	1.009	1.005
H28-N21	1.080	0.999	1.010	1.005
Angle [a]				
H28-N21-C11	117.1	117.49	112.2	112.7
H26-N24-C14	117.0	117.5	111.2	113.1
O19-C7-C8	123.7	124.05	122.1	123.7
O20-C10-C9	122.7	124.04	121.9	123.7
C1-C10-C14	177.2		155.0	137.7
C4-C7-C11	178.2		158.8	136.7
Charge				
N24&N21		-0.005	-0.383	-0.338
O19&O20		-0.368	-0.343	-0.345
H26&H28		0.146	0.204	0.262
Heat of formation [d]		-20.18	-41.31	-15.37
Dipole [e]		1.443	1.977	3.405

[a] Bond angles in degrees. [b] Bond lengths in angstroms. [c] MOPAC charge [d] Heat of formation in kcal mol^{-1}. [e] Dipole moment in Debyes. [f] R-factor is a measure of the agreement between the postulated structures relative to the diffractometer data collected.

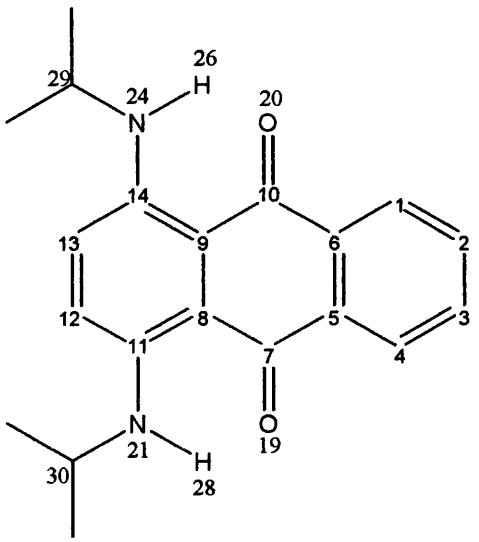
Table 5-9. Experimental vs. calculated geometries for 1,4-bis(isopropylamino)anthracene-9,10-dione[22] using the constrained AM1, PM3 and MNDO

Cambridge Database Reference Code BOCMUB	
R-Factor[f] 0.056	

Torsion	Cambridge	PM3[a]	AM1[a]	MNDO[b]
O20-C10-C9-C8	178.9	179.6	-179.9	-179.9
O19-C7-C8-C9	177.9	179.6	-179.9	-179.9
N21-C11-C8-C9	-178.4	180.0	180.0	180.0
N24-C14-C9-C8	179.3	180.0	180.0	180.0
H26-N24-C14-C9	179.3	0.0	0.0	0.0
H28-N21-C11-C8	-178.4	0.0	0.0	0.0
Distance				
O20H26	1.776	1.800	1.969	2.027
O19H28	1.747	1.801	1.969	2.027
N24-C14	1.362	1.399	1.377	1.396
N21-C11	1.351	1.400	1.377	1.396
N24-O20	2.595	2.583	2.684	2.743
N21-O19	2.586	2.583	2.684	2.743
C7-O19	1.256	1.231	1.247	1.234
C10-O20	1.260	1.231	1.247	1.234
C10-C9	1.461	1.480	1.474	1.505
C7-C8	1.464	1.479	1.474	1.505
C6-C10	1.480	1.488	1.479	1.503
C5-C7	1.496	1.487	1.479	1.503
H26-N24	1.080	1.009	0.955	0.997
H28-N21	1.080	1.009	0.955	0.997
Angle				
H28-N21-C11	117.1	117.2	119.9	118.7
H26-N24-C14	117.0	117.2	119.9	118.7
O19-C7-C8	123.7	122.1	124.1	122.2
O20-C10-C9	122.7	122.1	124.1	122.2
C1-C10-C14	177.2	177.5	178.2	178.4
C4-C7-C11	178.2	177.6	178.3	178.4
Charges				
N24&N21		-0.338	-0.005	-0.383
O19&O20		-0.345	-0.368	-0.343
H26&H28		0.262	0.146	0.204
Heat of formation		-32.71	-17.10	3.08
Dipole		1.945	2.807	1.986

[a]Molecules constrained in the Z plane at atoms 8,11,12,14,21,24,26,28,29 & 30.[b]Molecule constrained in the Z plane at atoms 7,8,10,11,12,14,21,24,26,28,29 & 30. Calculations carried out using keywords XYZ, Geo-ok and EF.

Table 5-10. Summary the computational data for 1,4-bis(isopropylamino)anthracene-9,10-dione



Method	Atoms constrained in Z plane [a]	N-H Bond	Structure	Nitrogen	Energy
PM3	No	Out of plane	Twisted	Tetrahedral	-41.30
PM3	C1,C2,C3,C4,C5,C6,C7,C8,C9,C10, C1,C12,C13,C14,O19,O20,N24,N21	Out of plane	Planar	Tetrahedral	-39.51
PM3	H26,N24,C14,C9,H28,N21,C11,C12	In plane	Planar	Tetrahedral	-38.06
PM3	C29H26,N24,C14,C9,C30,H28,N21, C11,C12	In plane	Planar	Planar	-32.79
AM1	No	Out of plane	Twisted	Tetrahedral	-20.18
AM1	C1,C2,C3,C4,C5,C6,C7,C8,C9,C10, C1,C12,C13,C14	In plane	Planar	Tetrahedral	-17.63
AM1	C29H26,N24,C14,C9,C30,H28,N21, C11,C12	In plane	Planar	Planar	-17.07
MNDO	No	Out of plane	Twisted	Tetrahedral	-15.37
MNDO	C1,C2,C3,C4,C5,C6,C7,C8,C9,C10, C1,C12,C13,C14,O19,O20,N24,N21	Out of plane	Planar	Tetrahedral	1.09
MNDO	H26,N24,C14,C9,H28,N21,C11,C12	In plane	Planar	Tetrahedral	1.69
MNDO	C29H26,N24,C14,C9,C30,H28,N21, C11,C12	In plane	Planar	Planar	3.08

[a]The input file for MOPAC is modified by freezing the position of the atoms in the Z plane for a particular atom

5.4.5 1,4-Bis(4-toluidinio)anthracene-9,10-dione

1,4-Bis(4-toluidinio)anthracene-9,10-dione again shows the same problems on calculation, as the alkylamino anthracene-9,10-diones with the nitrogen being pushed into a tetrahedral conformation and the molecule being buckled at the carbonyl groups (Table 5-11).

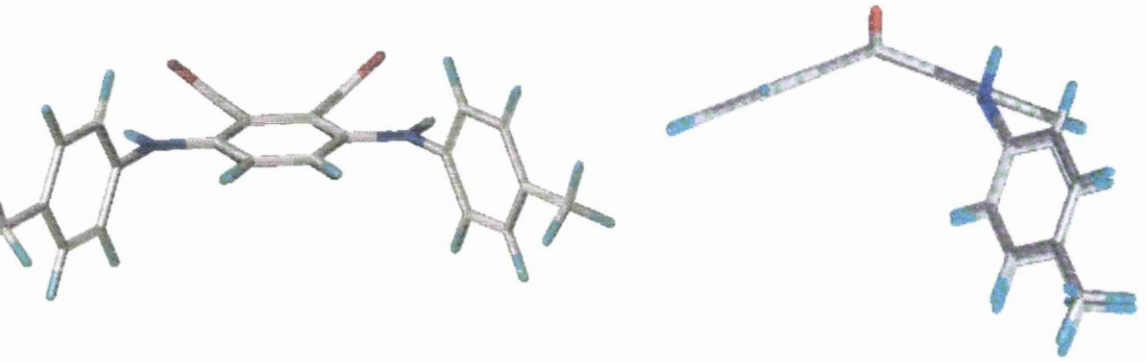


Figure 5-8. Predicted structure of 1,4-bis(4-toluidinio)anthracene-9,10-dione using MNDO

The AM1 method is further complicated as the nitrogen groups are predicted to be arranged in a staggered conformation.

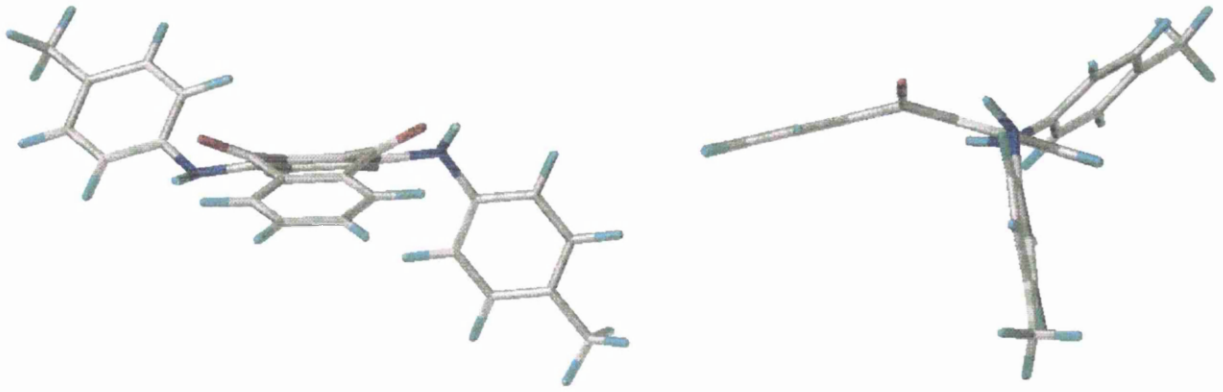


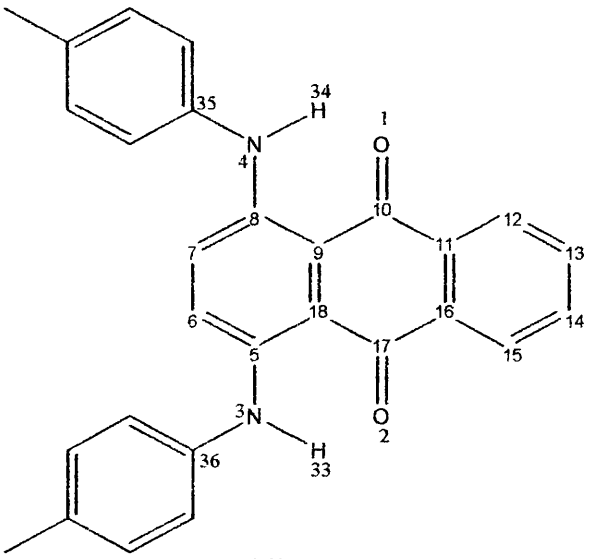
Figure 5-9. Predicted structure of 1,4-bis(4-toluidinio)anthracene-9,10-dione using AM1

The structural problem is again resolved by constraining the molecule in the z-plane (Table 5-12). This again leads to a difference in energy between the constrained and unconstrained

molecules. For the AM1 method this is 4.9 kcal mol^{-1}, for the PM3 method this is 7.1 kcal mol^{-1} and for the MNDO method this is 17.2 kcal mol^{-1} (Table 5-13).

Examination of the data for the constrained molecule shows that the PM3 method is most accurate at predicting the hydrogen bond length O1-H34 (Table 5-12) 1.822 Å compared to 1.882 Å for the CSD structure. However the AM1 method is more accurate at predicting the bond lengths of carbon-hetero atoms. For example the bonds O1-C17 and N3-C5 are predicted to be 1.247 Å, and 1.381 Å respectively, which compares to 1.254 Å and 1.369 Å respectively for the CSD structure (Table 5-12).

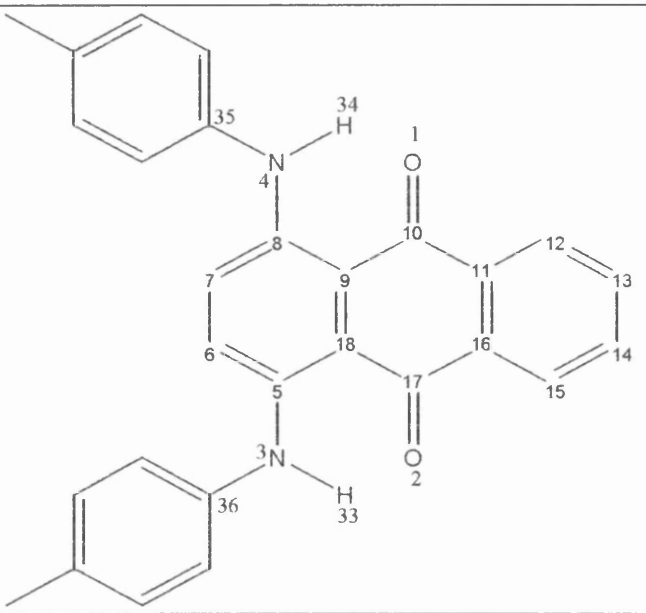
Table 5-11. Experimental vs. calculated geometries for 1,4-bis(4-toluidinio) anthracene-9,10-dione[22] using the unconstrained AM1, PM3 and MNDO methods

Cambridge Database Reference Code NOHTIN	
R-Factor [f] 0.056	

Torsion [a]	Cambridge	AM1	PM3	MNDO
H34-N4-C8-C9	8.4	-7.8	-4.7	-86.8
H33-N3-C5-C18	-13.1	-29.0	-16.1	85.4
N4-C8-C9-C18	177.0	174.2	-175.3	-176.2
N3-C5-C18-C9	177.0	175.7	175.9	176.2
O1-C10-C9-C18	-180.0	153.5	164.7	-135.3
O2-C17-C18-C9	178.5	151.5	-159.7	135.3
C8-C9-C10-C11	179.8	151.9	168.3	-137.2
C5-C18-C17-C16	179.4	151.4	-164.0	137.2
Distance [b]				
O1-H34	1.882	2.14	1.855	2.906
O2-H33	1.886	2.115	1.854	2.890
N4-C8	1.369	1.401	1.440	1.430
N3-C5	1.359	1.413	1.446	1.430
O1-C10	1.240	1.24	1.225	1.220
O2-C17	1.254	1.239	1.224	1.220
N3-H33	0.900	1.004	1.009	1.010
N4-H34	0.899	1.003	1.007	1.010
C9-C10	1.454	1.482	1.473	1.510
C18-C17	1.448	1.483	1.478	1.510
C10-C11	1.499	1.479	1.492	1.510
C17-C16	1.471	1.478	1.473	1.510
Angle [a]				
H34-N4-C8	116.1	115.2	111.7	110.3
H33-N3-C5	116.4	113.8	110.0	110.3
O1-C1O-C9	122.8	123.4	122.7	123.6
O2-C17-C18	123.7	123.5	122.5	123.6
N3-H33-O2	134.3	122.4	136.5	84.71
N4-H34-O1	132.8	123.5	132.3	85.82
C8-C10-C12	177.6	150.9	162.2	136.9
C5-C17-C15	177.0	152.0	165.7	137.0
Charges [c]				
O1&O2		-0.298	-0.328	-0.342
N4&N3		-0.251	0.064	-0.343
H34&H33		0.233	0.106	0.216
Heat of formation [d]		49.08	29.53	44.27
Dipole [e]		2.283	1.611	3.387

[a] Bond angles in degrees. [b] Bond lengths in angstroms [c] MOPAC charge [d] Heat of formation in kcal mol^{-1}. [e] Dipole moment in Debyes. [f] R-factor is a measure of the agreement between the postulated structures relative to the diffractometer data collected.[J]

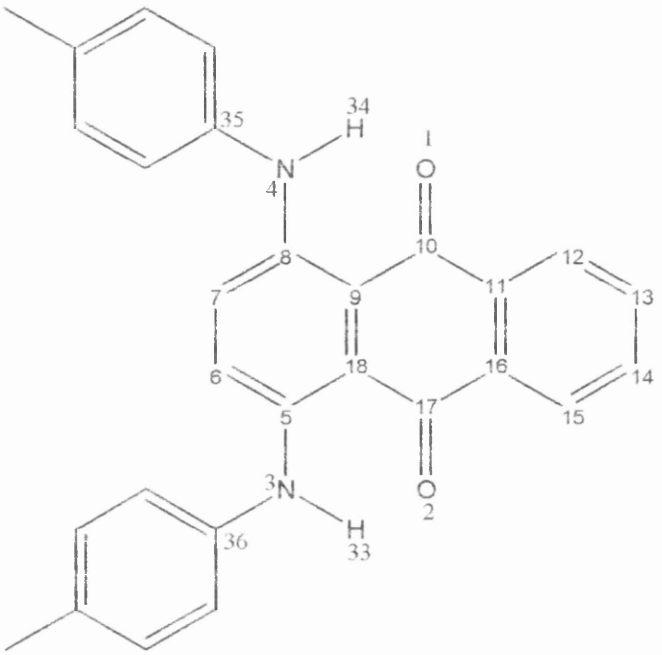
Table 5-12. Experimental vs. calculated geometries for 1,4-Bis(4-toluidinio) anthracene-9,10-dione[22] using the constrained AM1, PM3 and MNDO methods

Cambridge Database Reference Code NOHTIN	
R-Factor [f] =0.056	

Torsion [a]	Cambridge	AM1[g]	PM3[g]	MNDO[h]
H34-N4-C8-C9	8.4	0.8	0.7	0.8
H33-N3-C5-C18	-13.1	-0.8	-0.7	-0.8
N4-C8-C9-C18	177.0	-179.4	-179.6	-179.2
N3-C5-C18-C9	177.0	179.4	179.6	-179.2
O1-C10-C9-C18	-180.0	-179.9	-179.7	-179.6
O2-C17-C18-C9	178.5	179.9	179.7	179.6
C8-C9-C10-C11	179.8	179.7	-179.8	-179.7
C5-C18-C17-C16	179.4	179.7	179.9	179.7
Distance [b]				
O1-H34	1.882	1.981	1.822	2.073
O2-H33	1.886	1.981	1.822	2.073
N4-C8	1.369	1.381	1.401	1.396
N3-C5	1.359	1.381	1.401	1.396
O1-C10	1.240	1.247	1.231	1.234
O2-C17	1.254	1.247	1.231	1.234
N3-H33	0.900	0.966	1.007	0.999
N4-H34	0.899	0.967	1.007	0.999
C9-C10	1.454	1.474	1.477	1.504
C18-C17	1.448	1.474	1.477	1.504
C10-C11	1.499	1.478	1.488	1.503
C17-C16	1.471	1.478	1.488	1.503
Angle [a]				
H34-N4-C8	116.1	120.7	118.8	121.3
H33-N3-C5	116.4	120.7	118.8	121.3
O1-C1O-C9	122.8	123.8	121.7	122.0
O2-C17-C18	123.7	123.8	121.7	122.0
C8-C10-C12	177.6	178.0	177.4	178.1
C5-C17-C15	177.0	178.0	177.3	178.1
Charges [c]				
O1&O2		-0.334	-0.081	-0.342
N4&N3		-0.288	-0.459	-0.343
H34&H33		0.262	0.389	0.216
Heat of formation [d]		54.01	33.95	61.44
Dipole [e]		3.420	3.420	2.211

[a] Bond angles in degrees [b] Bond lengths in angstroms. [c] MOPAC charge [d] Heat of formation in kcal mol^{-1}. [e] Dipole moment in Debyes. [f] R-factor is a measure of the agreement between the postulated structures relative to the diffractometer data collected. [g] Molecule constrained in the Z plane at atoms 3,4,5,6,8,9,33,34,35 and 36. [h] Molecule constrained in the Z plane at atoms 3,4,5,8,9,10,17,18,33,34,35 and 36.

Table 5-13. Summary of the computational data for 1,4-bis(4-toluidinio) anthracene-9,10-dione



Method	Atoms constrained in the z-plane [a]	N-H Bond	Structure	Nitrogen	Energy
PM3	No	Out of plane	Twisted	Tetrahedral	26.89
PM3	C5,C5,C7,C8,C9,C10,C11,C12,C13,C14,C15,C16,C17,C18	Out of plane	Planar	Tetrahedral	27.98
PM3	C35,H34,N4,C8,C9,C6,C5,N3,H33,C36	In plane	Planar	Planar	33.94
AM1	No	Out of plane	Twisted	Tetrahedral	49.08
AM1	C5,C5,C7,C8,C9,C10,C11,C12,C13,C14,C15,C16,C17,C18	Out of plane	Planar	Tetrahedral	53.83
AM1	C35,H34,N4,C8,C9,C6,C5,N3,H33,C36	In plane	Planar	Planar	54.02
MNDO	No	Out of plane	Twisted	Tetrahedral	44.27
MNDO	C5,C5,C7,C8,C9,C10,C11,C12,C13,C14,C15,C16,C17,C18	Out of plane	Planar	Tetrahedral	50.72
MNDO	C35,H34,N4,C8,C9,C6,C5,N3,H33,C36	In plane	Planar	Planar	61.44

[a]The input file for MOPAC is modified by freezing the position of the atoms in the Z plane for a particular atom.

As the target molecules to be synthesised in these studies contain sulfur, it was necessary to checkout the accuracy of the AM1, PM3 and MNDO methods for predicting the geometries of the sulfur containing anthracene-9,10-dione systems. There are few examples of these systems in CSD with the exception of the well resolved structure of 1-(6-sulfanylhexylthio)anthracene-9,10-dione.

5.4.6 1-(6-Sulfanylhexylthio) anthracene-9,10-dione

The modelling of 1-(6-sulfanylhexylthio)anthracene-9,10-dione by AM1 produced a satisfactory overall geometry and gave a planar structure in line with the x-ray crystallographic data (Table 5-14).

Figure 5-10. 1-(6-sulfanylhexylthio)anthracene-9,10-dione predicted structure using the AM1 method

However, calculations using the PM3 and MNDO methods did not give a planar geometry, and the molecule was again bent at the carbonyl groups (Table 5-14).

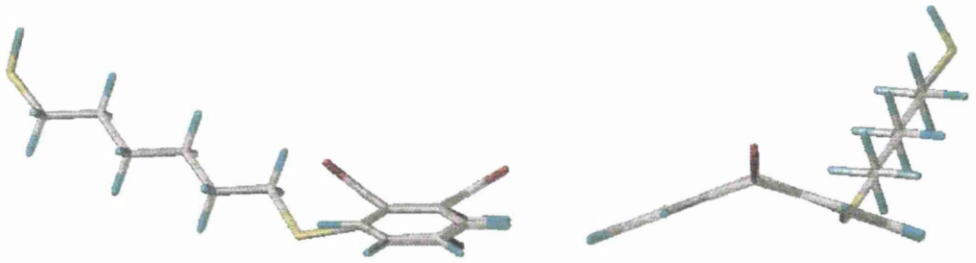
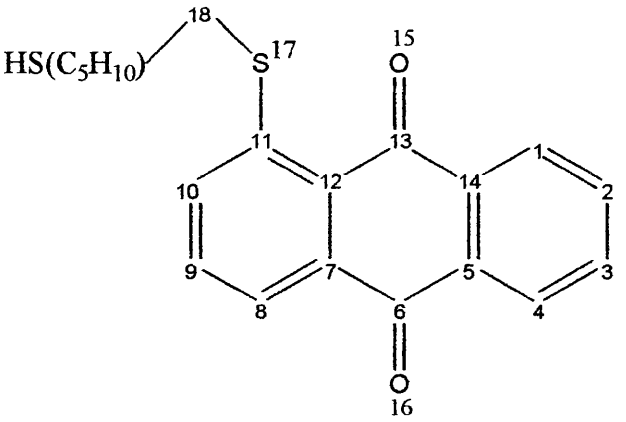


Figure 5-11. 1-(6-sulfanylhexylthio)anthracene-9,10-dione predicted structure using the MNDO method

The molecule was again constrained in the Z-plane to hold the heavy atoms in the same plane but this gave an energy penalty of 4.1 kcal mol^{-1} for PM3 and 9.0 kcal mol^{-1} for MNDO (Table 5-15).

Comparison of the CSD data with the three methods shows that the PM3 method is more accurate, in particular with the prediction of the bond lengths of S17-C11 and the O15-C13, which are respectively 1.773 Å and 1.218 Å for PM3, and 1.752 and 1.218 for the CSD structure.

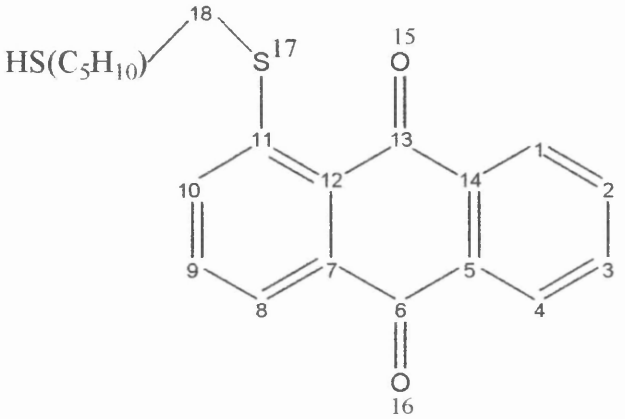
Figure 5-14. Experimental vs. calculated geometries for 1-(6-Sulfanylhexylthio) anthracene-9,10-dione[22] using the unconstrained AM1, PM3 and MNDO methods

Cambridge Database Reference Code WOGRIT01	
R-Factor [f] 0.044	

Distance [a]	Cambridge	AM1	PM3	MNDO
S17-C11	1.752	1.685	1.771	1.698
O15-C13	1.219	1.243	1.213	1.224
O16-C6	1.214	1.240	1.216	1.225
S17-O15	2.668	2.513	3.135	3.198
C12-C13	1.481	1.475	1.499	1.512
C7-C6	1.490	1.481	1.495	1.508
C13-C14	1.482	1.478	1.497	1.505
C5-C6	1.480	1.476	1.489	1.505
Torsion [b]				
S17-C11-C12-C7	-178.4	-179.9	176.7	171.1
O15-C13-C12-C7	-177.4	179.3	135.6	134.6
O16-C6-C7-C12	-175.0	-179.2	-152.2	-146.1
C18-S17-C11-C10	-1.0	0.5	22.9	-79.2
Angle [b]				
O15-C13-C12	121.2	121.4	124.1	123.7
O16-C6-C7	120.5	121.2	122.7	122.7
S17-C11-C12	121.3	118.4	120.9	122.1
C1-C11-C13	175.7	176.8	140.6	138.6
C4-C6-C8	175.3	176.8	147.1	143.4
C18-C17-C11	103.7	105.9	104.9	108.2
Charge [c]				
S17		0.380	0.093	0.111
O15		-0.277	-0.267	-0.266
016		-.0286	-0.295	-0.277
Dipole [e]		1.036	3.195	2.641
Heat of formation [d]		-28.20	-22.78	-34.32

[a] Bond lengths in angstroms. [b] Bond angles in degrees. [c] MOPAC charge [d] Heat of formation in kcal mol^{-1}. [e] Dipole moment in Debyes. [f] R-factor is a measure of the agreement between the postulated structures relative to the diffractometer data collected. Calculations carried out using keywords XYZ, Geo-ok and EF.

Figure 5-15. Experimental vs. calculated geometries for 1-(6-Sulfanylhexylthio) anthracene-9,10-dione[23] using the unconstrained for AM1 and constrained for PM3 and MNDO methods

Cambridge Database Reference Code WOGRIT01	
R-Factor [f] 0.044	

Torsion [b]	Cambridge	AM1	PM3[g]	MNDO[g]
S17-C11-C12-C7	-178.4	-179.9	-179.1	-177.4
O15-C13-C12-C7	-177.4	179.3	176.9	176.6
O16-C6-C7-C12	-175.0	-179.2	-176.0	-175.9
C18-S17-C11-C10	-1.0	0.5	-1.2	-0.5
Distance [a]				
S17-C11	1.752	1.685	1.773	1.693
O15-C13	1.219	1.243	1.218	1.228
O16-C6	1.214	1.240	1.221	1.230
S17-O15	2.668	2.513	2.781	2.728
C12-C13	1.481	1.475	1.498	1.512
C7-C6	1.490	1.481	1.495	1.509
C13-C14	1.482	1.478	1.493	1.507
C5-C6	1.480	1.476	1.483	1.499
Angle [b]				
O15-C13-C12	121.2	121.4	122.3	121.8
O16-C6-C7	120.5	121.2	121.0	120.6
S17-C11-C12	121.3	118.4	122.0	120.2
C1-C11-C13	175.7	176.8	175.3	175.5
C4-C6-C8	175.3	176.8	175.8	175.7
C18-C17-C11	103.7	105.9	106.7	112.7
Charge [c]				
S17		0.380	0.122	0.183
O15		-0.277	-0.280	-0.274
016		-.0286	-0.311	-0.296
Dipole [e]		1.036	1.910	1.110
Heat of formation [d]		-28.20	-18.71	-25.34

[a] Bond lengths in angstroms. [b] Bond angles in degrees. [c] MOPAC charge [d] Heat of formation in kcal mol^{-1}. [e] Dipole moment in Debyes. [f] R-factor is a measure of the agreement between the postulated structures relative to the diffractometer data collected. [g] Molecules constrained in the Z plane at atoms 5,6, 7,11,12,13,14,17 & 18. Calculations carried out using keywords XYZ, Geo-ok and EF.

5.4.7 Conclusion

Examination of the data shows that MNDO method is the least accurate method for the amino and sulfur anthracene-9,10-dione derivatives, in terms of the intramolecular hydrogen bond lengths and hetero atom carbon bond lengths and the general shape. The PM3 method is the most accurate at predicting the intramolecular hydrogen bond lengths, the heteroatom carbon bond lengths (i.e. C11-S17 and O15-C13) in the sulfur anthracene-9,10-dione derivative but is poor at predicting the general planar shape of both the amino and sulfur anthracene-9,10-dione derivatives. The AM1 method is not as accurate at predicting the hydrogen bond lengths in comparison to the PM3 method but it is much better than the MNDO method. It is also better at predicting the heteroatom carbon bond lengths in the amino derivatives for example Table 5-12 shows the bond length N4-C8 which is 1.369 Å for the CSD structure, the AM1 method predicts this bond length to be 1.381 Å and the length predict by the PM3 method is worse, with a value of 1.401 Å. The AM1 method also predicts a good general shape for the sulfur derivatives i.e. planar, which is agreement with the CSD data.

It is widely believed that the mode of action of anthracene-9,10-dione based anti-cancer agents is due to the ability of the planar molecule to interact with DNA. It is therefore desirable that any computational method used to model these molecules should predict a structure which is planar. As the AM1 method does correctly predict the sulfur anthracene-9,10-dione derivative will be planar without any constraints on the optimisation, it was selected in preference to the other two methods and was used to model all the sulfur containing molecules synthesised in this work

5.5 *Ab initio* calculations

Following the results obtained from the semi-empirical calculations it was decided to run a number of reference *ab initio* calculations. Due to the increased processor time required for the latter calculations as compared to the former, 1,4-bis(methylamino)anthracene-9,10-dione,

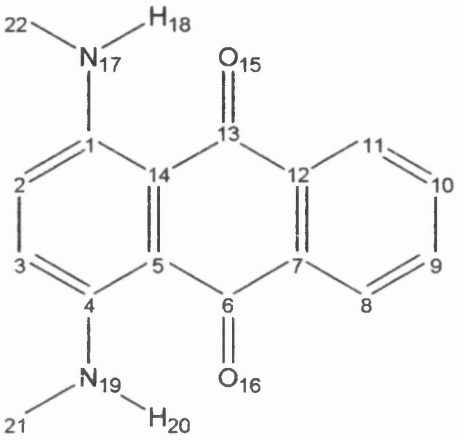
was selected as the simplest derivative of the amino-anthracene-9,10-diones that didn't remain planar when modelled using the semi-empirical methods. The simplest basis set, STO-3G, was selected to give an idea of the time scale.

The computational time required to complete a geometry optimisation of 1,4-bis(methylamino)anthracene-9,10-dione using the PM3 method was 14 minutes (using a Silicon Graphics Indy R5000), but a comparable optimisation of the same molecule using the STO-3G basis set took 36 hours (using a direct SCF). In the latter however, the molecule remains planar in line with x-ray crystallographic data. The STO-3G basis set is a minimal basis set and has several drawbacks, which include the inability to properly represent non-spherical electron distributions, found in p orbitals. To overcome this problem higher level split basis sets are required such as the 3-21G basis set. 1,4-Bis(methylamino)anthracene-9,10-dione was therefore modelled using 3-21G basis set and the 6-31G** basis set. In the latter more basis functions per atom are included, such as d-orbitals on heavy atoms and p orbitals on hydrogen: these are known as polarisation functions which allow distortion of electron density across the molecule.

However the run time for 6-31G** calculation on 1,4-bis(methylamino)anthracene-9,10-dione was three hundred and ninety six hours.

Although the STO-3G basis set is a minimal basis set it does actually produce some very good results. In particular the carbon nitrogen bond C1-N17, this is predicted to be 1.359 Å, which compared to 1.359Å for the CSD structure and 1.401 Å for the higher level 3-21G basis set, is an excellent result. The AM1 method also fairs well in comparison to the ab initio methods, giving very reasonable results with the exception of the intramolecular hydrogen bond distance. In particular the O15-C13 bond length of 1.240 Å compared to a CSD with a value of 1.254 Å (Table 5-16).

Table 5-16. 1,4-bis (methylamino) anthracene-9,10-dione modelled using ab initio methods, in comparison with CAMJOP CSD structure and the computed AM1 structure.



	CAMJOP CSD	CAMJOP AM1	STO-3G	3-21G	6-31G**
Distance [a]					
C1N17	1.359	1.376	1.359	1.401	1.361
C4N19	1.347	1.387	1.359	1.401	1.361
H18N17	0.805	0.999	0.999	1.017	0.991
H20N19	0.930	1.000	0.999	1.017	0.991
O15H18	1.781	1.949	1.812	1.748	1.860
O16H20	1.899	1.956	1.812	1.748	1.860
O15C13	1.254	1.240	1.233	1.234	1.210
O16C6	1.255	1.220	1.233	1.234	1.210
C6C5	1.449	1.486	1.465	1.516	1.478
C12C13	1.480	1.476	1.482	1.511	1.478
Angle [b]					
H18N17C1	110.2	118.7	115.9	114.7	116.2
H20N19C4	110.4	1119.4	115.9	114.7	116.2
O15C13C14	123.1	124.7	123.4	123.1	122.9
O16C6C5	122.4	124.7	123.4	123.1	122.9
C11C13C1	176.3	178.2	177.7	176.3	177.7
C8C6C4	176.0	178.0	177.7	176.3	177.7
Torsion [b]					
H18N17C1C14	-9.6	180.0	0.0	0.4	0.0
H20N19C4C5	-1.2	180.0	0.0	0.5	0.0
O15C13C14C5	177.7	180.0	180.0	179.9	180.0
O16C6C5C12	-178.9	180.0	180.0	179.9	180.0
Dipole [c]		3.077	2.361	0.324	2.004
Total energy [d]			-544563.3	-540760.7	-547590.6
HOMO [e]					-6.808
LUMO [e]					0.971

[a] Bond lengths in angstroms. [b] Bond angles in degrees. [c]. Dipole moment in Debyes [d] In A.U.. [e] In eV

5.6 Calculated properties

The AM1 method was selected to model the sulfur containing anthracene-9,10-diones. The following calculated properties were selected to explore the likely activity of the molecules synthesised.

1. *Energy of the Highest Occupied Molecular Orbital (E_{HOMO}).* The E_{HOMO} or ionization potential (eV) is the energy required to remove an electron from the molecule. It should give an indication of the molecules ability; to undergo a one electron oxidation; the lower in energy the HOMO is the easier it would be to loose an electron. If the mode of action involves an oxidation step, then the HOMO should reflect this.
2. *Energy of the Lowest Unoccupied Molecular Orbital (E_{LUMO});* The E_{LUMO} (eV) can also be thought of as the compound's electron affinity. It should give an indication of the molecules ability to undergo a one electron reduction; the lower in energy the LUMO is the easier it would be for the molecule to accept an electron. It is thought that the ability of anthracene-9,10-diones to undergo this one electron reduction is why anthracene-9,10-dione based drugs are cardiotoxic (see section 1.2.3.).
3. *Atomic charges* on the nitrogen, oxygen, sulphur and hydrogen atoms are the most significant in the molecule as they would be the mostly likely sites of interaction and bonding with other cellular molecules such as DNA. The larger the charge on these atoms the more likely they are to interact with DNA and form non-covalent bonds.
4. *Dipole moment (μ).* The dipole moment of a polyatomic molecule is the vector sum of all the charges acquired on its atoms due to the differences in their respective electronegativities[24]. Compounds with high dipole moment moments are often referred to as "polar" and are generally soluble in polar solvents such as water and hence the aqueous cell environment. Less polar compounds are generally more stable in lipophilic solvents. The measurements were recorded in Debyes.
5. *Hetero atom bond lengths.* These are important as the hetero atoms are the likely binding sites and distortion in the bond lengths will affect how the molecule would bind to other cellular molecules. Hydrogen bond length may also play a part in binding.

5.6.1 Active vs. inactive derivatives of anthracene-9,10-dione

Looking at the calculated properties of all the synthesised molecules it is clear that there is a wide distribution of charge across the anthracene-9,10-dione molecule (Table 5-17). In order to assess the likely binding properties of these derivatives, it is necessary to compare these results with those obtained for the highly active derivatives such as Mitoxantrone **(24b)** and Amentantrone **(24a)** on the one hand, with those for the inactive anthracene-9,10-dione **(1)** and 1,4-dihydroxyanthracene-9,10-dione **(111)** on the other. Accordingly the molecules were constructed using Sybyl and optimised using the AM1 method with the appropriate constraints as before (Table 5-17).

An analysis of the calculated results show that in Mitoxantrone **(24b),** the oxygen atoms O15 and O16 carry negative charges of -0.39, the nitrogen atoms N17 and N19 also carry a negative charges of -0.34, while the hydrogen atoms H18 and H20 carry a positive charge of 0.26.

Comparison of the active compounds Amentantrone (24a) and Mitoxantrone (24b).

Bond lengths and charge:

The bond length is slightly longer in the case of Mitoxantrone **(24b)** with a value of 1.375Å compared to 1.397 Å for Amentantrone **(24a)**, the O15-C13 bond length is also longer with values of 1.252 Å for Mitoxantrone **(24b)** and 1.240 Å for Amentantrone **(24a)** (Table 5-17). The atomic charges on the nitrogen atoms N17 and N19 are more negative for Mitoxantrone **(24b)** with average value of -0.34 compared to -0.30 for Amentantrone **(24a)**. A similar effect is found for the charge on the oxygen atoms O15 and O16 with values of -0.38 for Mitoxantrone **(24b)** compared to -0.31 for Amentantrone **(24a)**. The hydrogen atoms H23 and H24 are more positive with values of 0.26 for Mitoxantrone **(24b)** compared to 0.23 for Amentantrone **(24a)**. The significance of the dipole moment is unclear as it will be highly affected by the orientation of any side chain present and therefore cannot be considered, except to note perhaps that the higher the value the more polar the molecule.

Energies:

The HOMO or ionisation potential for Mitoxantrone **(24b)** is much lower than Amentantrone **(24a)** with values of 7.71 eV and 7.95 eV respectively. This equates to an energy difference of 5.5 kcal mol^{-1} (1 eV is equal to 23.06 kcal mol^{-1}) it is therefore more difficult, for Amentantrone **(24a)** to lose an electron than Mitoxantrone **(24b)**. This means Mitoxantrone **(24b)** will be more likely to transfer an electron to DNA and form a stable polar complex.

The parameterisation of the AM1 method produces negative values for the LUMO energies. The LUMO of Mitoxantrone **(24b)** is -1.29 eV and -1.04 eV for Amentantrone **(24a)**. This is clearly incorrect and is an artefact of the original parameterisation of the AM1 method developed by Dewar. More accurate calculations at the ab initio level have been carried out on sulfur containing species (see latter), which confirm that the LUMO energy is positive and overestimated by the AM1 method by at least 2 eV. Accordingly it was decide to adjust the LUMO energies calculated by the AM1 method by 2 eV, to give positive numbers for comparative purposes

The adjusted values of Mitoxantrone **(24b)** and Amentantrone **(24a)** are 0.71 eV and 0.96 eV This means that Mitoxantrone **(24b)** will except an electron more easily and is therefore more likely to undergo a one electron reduction.

Comparison of the inactive compounds anthracene-9,10-dione and 1,4-dihydroxyanthracene-9,10-dione (Table 5-17).

Bond lengths and charge:

The bond lengths are similar for anthracene-9,10-dione **(1)** and 1,4-dihydroxyanthracene-9,10-dione **(111)** for example the O15-C13 bond is 1.240 Å for anthracene-9,10-dione **(1)** and 1.246 Å for 1,4-dihydroxyanthracene-9,10-dione **(111).** The atomic charge on the oxygen

atoms O15 and O16 is more negative for 1,4-dihydroxyanthracene-9,10-dione (**111**) than anthracene-9,10-dione (**1**) with values of -0.33 and -0.31 respectively.

Energies:

The ionisation potential for anthracene-9,10-dione (**1**) is higher than 1,4-dihydroxyanthracene-9,10-dione (**111**) with values of 10.12 eV and 9.07 eV respectively. The LUMO for 1,4-dihydroxyanthracene-9,10-dione (**111**) has an adjusted value of 0.38 eV compared to an adjusted value of 0.61 eV for anthracene-9,10-dione (**1**).

Comparison of active vs. inactive anthracene-9,10-diones

The bond lengths of the carbon-hetero atoms are not significant, as both Mitoxantrone (**24b**) and Amentantrone have very similar bond lengths to 1,4-dihydroxyanthracene-9,10-dione and anthracene-9,10-dione (**1**). The presence of negative oxygen atoms O21 or O22 in 1,4-dihydroxyanthracene-9,10-dione (**111**) and Mitoxantrone (**24b**) does not confer activity nor do the acidic hydrogen atoms H23 and H24. The presence of the basic nitrogen atoms N17 and N19 in Mitoxantrone (**24b**) and Amentantrone (**24a**) however may be significant. Although both the oxygen atoms O21 and O22 of 1,4-dihydroxyanthracene-9,10-dione (**111**) and the nitrogen atoms N17 and N19 of Mitoxantrone (**24b**) have lone pair of electrons, this doesn't confer activity, it appears that the atoms need to carry a larger negative atomic charge. The nitrogen atoms N17 and N19 of Amentantrone (**24a**) are significantly more negative than the oxygen atoms O22 and O21 of 1,4-dihydroxyanthracene-9,10-dione with values of -0.30 and -0.26 respectively.

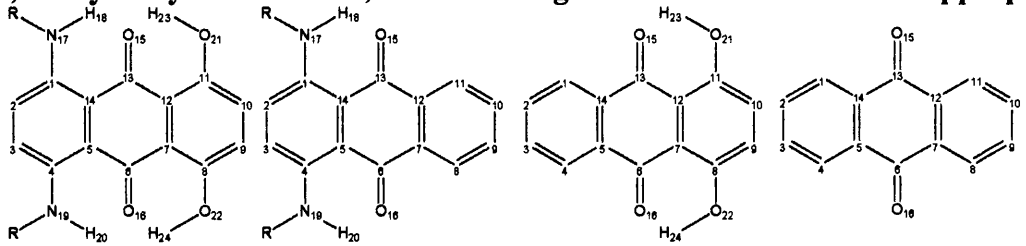
The ionisation potential does produce a significant pattern which correlates to the activity of the molecules in Table 5-17. Mitoxantrone (**24b**) has the highest activity followed by Amentantrone (**24a**) with 1,4-dihydroxyanthracene-9,10-dione (**111**) and anthracene-9,10-dione (**1**) being inactive. This activity is reflected in the ionisation potentials of 7.71 eV, 7.95 eV, 9.07 eV and 10.1 eV for the four molecules. There is a difference of at least 1 eV

between the active and inactive derivatives, which in terms of energy is 23.1 kcal mol^{-1}. This is a significant energy difference and suggest that oxidation of the molecules may be related to activity. In support of this, it has been reported that Mitoxantrone **(24b)** is oxidised in situ by cytochrome P-450 and the inhibition of this reaction leads to a complete loss in the cytotoxicity of Mitoxantrone **(24b),** in HepG2 cells[25].

The LUMO does not appear to correlate with activity, Amentantrone **(24a)** has the largest adjusted energy of 0.96 eV followed by Mitoxantrone **(24b)** with an adjusted value of 0.71 eV, then anthracene-9,10-dione **(1)** with an adjusted value of 0.61 eV and finally 1,4-dihydroxyanthracene-9,10-dione **(111)** which has an adjusted value of 0.38 eV.
It may be significant in the cardiotoxicity of the molecules as a high LUMO could indicate a reduced ability to form free radicals.

The presence of a nitrogen or oxygen atom in the side chain of Mitoxantrone **(24b)** and Amentantrone **(24b)** is also important, as both would improve solubility in the cellular environment. The basic nitrogen atom would stabilise interaction with DNA and promote binding[26]. These studies however were not designed to test this hypothesis and are more concerned with the effect of sulphur on the anthracene-9,10-dione molecule.

Table 5-17. Comparison of computational data for the highly active 1,4-bis-(aminoalkyl)anthracene-9,10-diones vs. the inactive anthracene-9,10-dione and 1,4-dihydroxyanthracene-9,10-dione using the AM1 method and the appropriate constraints



(24b) R=$(CH_2)_2NH(CH_2)_2OH$ (24a) (111) (1)

	Mitoxantrone	Amentantrone	Anthracene-9,10-dione	1,4-Dihydroxy anthracene -9,10-dione
Number	**(24b)**	**(24a)**	**(1)**	**(111)**
Distance [a]				
C11O21	1.390	N/A	N/A	1.366
N17-C1	1.375	1.397	N/A	N/A
H23O21	0.960	N/A	N/A	0.970
O15C13	1.252	1.240	1.240	1.246
Charge [b]				
H23	0.260	N/A	N/A	0.262
H24	0.259	N/A	N/A	0.262
O21	-0.262	N/A	N/A	-0.257
O22	-0.263	N/A	N/A	-0.257
O15	-0.387	-0.310	-0.309	-0.331
O16	-0.381	-0.315	-0.314	-0.331
N17	-0.338	-0.294	N/A	N/A
N19	-0.334	-0.301	N/A	N/A
H18	0.262	0.221	N/A	N/A
H20	0.262	0.234	N/A	N/A
HOMO [c]	-7.780	-7.952	-10.124	-9.071
Dipole moment [d]	5.548	1.631	0.001	1.787
LUMO 1 [c]	-1.291	-1.038	-1.393	-1.616
LUMO 2 [e]	0.709	0.962	0.607	0.384

[a] Bond lengths in angstroms. [b] Mopac charge [c] In eV. [d] Dipole moment in Debyes. [e] In eV, a factor of 2 eV has been added to make the LUMO energies positive.

5.6.2 1,4-Bis-(amino)-5,8-dichloroanthracene-9,10-diones

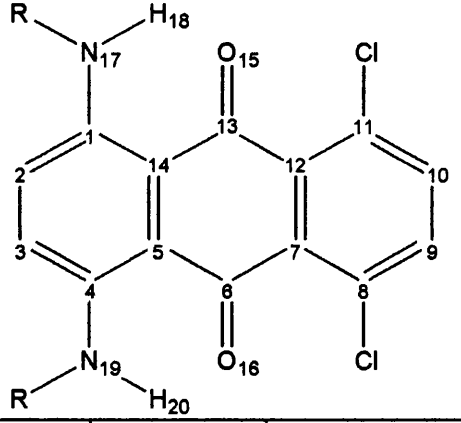
The atomic charge on the nitrogen atoms N17 and N19 for all the chloro derivatives (**123, 124, 125, 127,128, 157**) is in the region of -0.34 which is comparable to Mitoxantrone (**24b**) which has a value of -0.34 but greater than Amentantrone (**24b**) which has a value of -0.30 (Table 5-18).

The ionisation potential for the all the chloro derivatives is lower by comparison with Amentantrone (**24a**). The highest ionisation potential being 7.83 eV for 1,4-bis-[2-(hydroxyethyl)amino]-5,8-dichloroanthracene-9,10-dione (**125**). Some of the derivatives such as 1,4-bis(propylamino)-5,8-dichloroanthracene-9,10-dione (**123**) have even lower values than Mitoxantrone (**24b**), with values of 7.59 eV and 7.71 eV respectively (Table 5-18). The ionisation potentials for the chloro derivatives are closer to the active molecules Mitoxantrone (**24b**) and Amentantrone (**24a**) rather than the inactive 1,4-dihydroxyanthracene-9,10-dione (**111**) and anthracene-9,10-dione (**1**), which have ionisation potentials of 10.12 eV and 9.07 eV respectively.

The effect on the LUMO is a reduction to 0.76 eV for 1,4-bis{[2-(hydroxyethyl)amino)ethyl]amino}-5,8-dichloroanthracene-9,10-dione (**157**), from a corrected value of 0.96 eV for Amentantrone (**24a**) but this value is higher than Mitoxantrone (**24b**) which has a corrected value of 0.71 eV (Table 5-18).

The presence of chlorine in the anthracene-9,10-dione ring results in a decrease in the ionisation potential suggesting that the chlorine containing molecule are more likely to transfer an electron to DNA and form a stable polar complex than Mitoxantrone (**24b**). The potential negative effect of chlorination is a decrease in the energy of the LUMO which suggests that this molecule could more easily form free radical species, however the values are comparable to Mitoxantrone (Table 5-18).

Table 5-18. Calculated properties of selected 1,4-bis(amino)-5,8-dichloroanthracene-9,10-diones using the AM1 method



R	$(CH_2)_2CH_3$	$CH_2CH(CH_3)_2$	$(CH_2)_2OH$	C_6H_5	$(CH_2)_2N(CH_3)_2$	$(CH_2)_2N(C_2H_5)_2$	$(CH_2)_2N(CH_2)_2OH$	Amentantrone
	(123)	(124)	(125)	(131)	(127)	(128)	(157)	(24a)
Distance [a]								
C1N17	1.374	1.378	1.376	1.383	1.375	1.374	1.375	1.397
C4N19	1.382	1.396	1.384	1.379	1.383	1.383	1.384	1.391
H18N17	0.997	1.002	0.996	0.996	0.996	0.996	0.995	1.000
H20N19	0.995	1.022	0.994	0.997	0.994	0.994	0.994	1.000
O15H18	1.939	2.065	1.939	1.967	1.941	1.942	1.953	2.098
O16H20	1.924	1.971	1.922	1.962	1.924	1.923	1.934	2.042
O15C13	1.244	1.243	1.244	1.243	1.244	1.242	1.244	1.240
O16C6	1.242	1.241	1.242	1.244	1.242	1.244	1.242	1.242
Charge [b]								
O15	-0.321	-0.303	-0.319	-0.319	-0.319	-0.320	-0.323	-0.310
O16	-0.317	-0.306	-0.316	-0.320	-0.316	-0.316	-0.318	-0.315
N17	-0.343	-0.346	-0.339	-0.311	-0.342	-0.340	-0.339	-0.294
N19	-0.346	-0.322	-0.343	-0.377	-0.345	-0.345	-0.345	-0.301
H18	0.264	0.251	0.265	0.275	0.265	0.264	0.263	0.221
H20	0.264	0.243	0.265	0.275	0.264	0.264	0.263	0.234
HOMO [c]	-7.593	-7.737	-7.826	-7.808	-7.681	-7.641	-7.637	-7.952
Dipole moment [d]	2.505	1.911	1.706	2.936	2.421	2.742	4.128	1.631
LUMO 1 [c]	-1.234	-1.184	-1.394	-1.276	-1.289	-1.261	-1.239	-1.038
LUMO 2 [e]	0.766	0.816	0.606	0.724	0.711	0.739	0.761	0.962

[a] Bond lengths in angstroms. [b] Mopac charge [c] In eV. [d] Dipole moment in Debyes. [e] In eV, a factor of 2 eV has been added to make the LUMO energies positive

5.6.3 1,4-bis(amino)-5,8-(sulfanyl)anthracene-9,10-diones

As the presence of hydroxyl groups attached to the 5- and 8- positions of the anthracene-9,10-dione moiety has such a significant effect on the physical and biological properties, it was desirable to model the equivalent sulfur derivatives of Mitoxantrone, 1,4-bis{[2-(hydroxyethyl)amino)ethyl]amino}-5,8-bis(thiol)anthracene-9,10-dione (**158**). The introduction of a sulfur atom in place of an oxygen atom in 1,4-bis{[2-(hydroxyethyl)amino)ethyl]amino}-5,8-bis(thiol)anthracene-9,10-dione (**158**) does not effect the charge on the nitrogen atoms N17 and N19, with values of -0.34, which is the same value calculated for Mitoxantrone (**24b**) (Table 5-19).

The LUMO is unaffected by the introduction of sulfur, as 1,4-bis{[2-(hydroxyethyl)amino)ethyl]amino}-5,8-bis(thiol)anthracene-9,10-dione (**158**) has a adjusted value of 0.70 eV compared to a adjusted value of 0.71 eV for Mitoxantrone (**24b**). The ionisation potential is lowered very slightly to 7.69 eV for 1,4-bis{[2-(hydroxyethyl)amino)ethyl]amino}-5,8-bis(thiol)anthracene-9,10-dione (**158**) from 7.71 eV for Mitoxantrone (**24b**) (Table 5-19).

Introduction of other sulfur substituents has a similar effect, examination of the data for 1,4-bis{[2-(hydroxyethyl)amino)ethyl]amino}-5,8-bis(ethylsulfanyl)anthracene-9,10-dione (**161**) and 1,4-bis{[2-(hydroxyethyl)amino)ethyl]amino}-5,8-bis(phenylsulfanyl)anthracene-9,10-dione (**162**) shows the LUMO is increased in comparison to Mitoxantrone with adjusted values of 0.91 eV for 1,4-bis{[2-(hydroxyethyl)amino)ethyl]amino}-5,8-bis(ethylsulfanyl)anthracene-9,10-dione (**161**) and 0.90 eV for 1,4-bis{[2-(hydroxyethyl)amino)ethyl]amino}-5,8-bis(phenylsulfanyl)anthracene-9,10-dione (**162**) compared to 0.71 eV for Mitoxantrone (**24b**) (Table 5-19). The ionisation potential of 7.71 eV for Mitoxantrone (**24b**) is also reduced in the sulfur derivatives 1,4-bis{[2-(hydroxyethyl)amino)ethyl]amino}-5,8-bis(ethylsulfanyl)anthracene-9,10-dione (**161**) and 1,4-bis{[2-(hydroxyethyl)amino)ethyl]amino}-5,8-bis(phenylsulfanyl)anthracene-9,10-dione (**162**) with values of 7.31 eV and 7.34 eV respectively (Table 5-19). Again the values

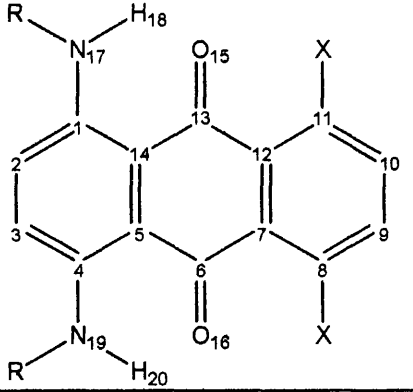
obtained for the sulfur containing systems are closer to the active compounds Mitoxantrone **(24b)** and Amentantrone **(24a)** than the inactive compounds 1,4-dihydroxyanthracene-9,10-dione (**111**) and anthracene-9,10-dione **(1).**

The data shows the modification of the groups at the 5- and 8- positions can have a marked effect. Introduction of SH in place of OH has no real effect however the introduction of sulfur substituents such as SEt and SPh leads to a decreased ionisation potential which could indicate improved activity.

This general pattern of reduced ionisation potential and increased LUMO energy is also seen in the other derivatives. The synthesised derivatives have similar physical properties to the active derivatives such as Amentantrone **(24b)** as opposed to the inactive derivatives such as anthracene-9,10-dione (**1**). For example 1,4-bis[(2-(hydroxyethyl)amino]-5,8-bis(ethylsulfanyl)anthracene-9,10-dione **(142)** has an atomic charge of -0.35 for the nitrogen atoms and an ionisation potential of 7.44 eV which compares to -0.34 and 7.71 eV for Mitoxantrone. 1,4-Bis[(2-(hydroxyethyl)amino]-5,8-bis(ethylsulfanyl)anthracene-9,10-dione **(142)** has a LUMO value of 0.93 eV and compares to 0.79 eV for Mitoxantrone**(24b).** The sulfur containing molecules synthesised in this work **(148, 133 & 134)** were also calculated at the ab initio 6-31G** level. The results are consistent with the AM1 values shown in Table 5-21, taking 1,4-bis(propylamino)-5,8-bis(ethylsulfanyl)anthracene-9,10-dione (**133**) as the example it can be seen that the charge distributions are magnified and the HOMO energies are around 1 eV smaller. The calculated charge on the nitrogen atom is -0.34 in the AM1 method (Table 5-20) which compares to -0.83 using the ab initio method 6-31G** (Table 5-21), and the calculated HOMO is -7.35 eV for the AM1 method compared to -6.63 eV for the ab initio method. Significantly however the LUMO energies are positive in line with the adjusted values. 1,4-bis(propylamino)-5,8-bis(ethylsulfanyl)anthracene-9,10-dione (**133**) has an adjusted LUMO of 0.96 eV for the AM1 method compared to 1.05 eV for the ab initio method 6-31G**.

The hetero-carbon bond lengths calculated by the AM1 method compare well to the ab initio method 6-31G**. The C1-N17 for 1,4-bis(propylamino)-5,8-bis(ethylsulfanyl)anthracene-9,10-dione (**133**) is predicted to be 1.363 Å by the 6-31G** ab initio compared to 1.376 Å for the AM1 method. The S22-C11 bond also compares well with values of 1.788 Å for the 6-31G** ab initio method compared to 1.779 Å for the AM1 method.

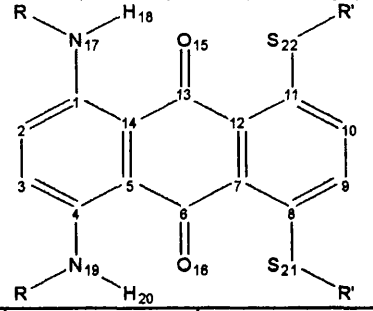
Table 5-19. Calculated properties of selected 1,4-bis(amino)-5,8-bis(sulfanyl)anthracene-9,10-diones using the AM1 method



R	CH_3	CH_3	$(CH_2)_2$ NH $(CH_2)_2OH$	$(CH_2)_2$ NH $(CH_2)_2OH$	$(CH_2)_2$ NH $(CH_2)_2OH$	$(CH_2)_2$ NH $(CH_2)_2OH$	$(CH_2)_2$ NH $(CH_2)_2OH$	$(CH_2)_2CH_3$	$CH_2CH(CH_3)_2$	$(CH_2)_2OH$	C_6H_5
X	OH	SH	SH	OH	H	S CH_2 CH_3	S C_6H_5	H	H	H	H
	(159)	(160)	(158)	(24b)	(24a)	(161)	(162)	(150)	(163)	(149)	(164)
Distance [a]											
C1N17	1.378	1.378	1.375	1.375	1.397	1.376	1.375	1.376	1.380	1.377	1.386
H18N17	0.994	0.994	0.995	0.955	1.000	0.994	0.996	0.997	1.003	0.997	1.004
O15H18	1.959	1.958	1.953	1.960	2.098	1.968	1.968	1.968	2.037	1.970	1.878
O15C13	1.253	1.249	1.248	1.252	1.240	1.253	1.253	1.249	1.244	1.245	1.247
Charge [b]											
O15	-0.390	-0.381	-0.368	-0.387	-0.310	-0.328	-0.336	-0.343	-0.325	-0.341	-0.335
O16	-0.390	-0.381	-0.372	-0.381	-0.315	-0.325	-0.334	-0.336	-0.315	-0.334	-0.335
N17	-0.335	-0.336	-0.341	-0.338	-0.294	-0.343	-0.341	-0.342	-0.345	-0.342	-0.278
N19	-0.335	-0.336	-0.345	-0.334	-0.301	-0.347	-0.346	-0.347	-0.343	-0.343	-0.278
H18	0.265	0.264	0.262	0.262	0.221	0.262	0.264	0.262	0.249	0.264	0.262
H20	0.265	0.265	0.263	0.262	0.234	0.263	0.262	0.262	0.252	0.263	0.262
X [c]	-0.263	0.145	0.149	-0.262	N/A	0.375	0.495	N/A	N/A	N/A	N/A
X [c]	-0.263	0.144	0.108	-0.263	N/A	0.369	0.496	N/A	N/A	N/A	N/A
HOMO [d]	-7.706	-7.706	-7.687	-7.706	-7.952	-7.314	-7.336	-7.567	-7.591	-7.799	-7.734
Dipole moment [e]	4.464	4.225	5.371	5.548	1.631	2.897	2.986	2.271	3.069	1.288	0.237
LUMO 1 [d]	-1.319	-1.260	-1.296	-1.291	-1.038	-1.085	-1.095	-1.066	-0.944	-1.228	-1.451
LUMO 2 [e]	0.681	0.740	0.704	0.709	0.962	0.915	0.905	0.934	1.056	0.772	0.549

[a] Bond lengths in angstroms. [b] Mopac charge [c] Exocyclic hetero atom [d] In eV. [e] Dipole moment in Debyes. [e] In eV, a factor of 2 eV has been added to make the LUMO energies positive

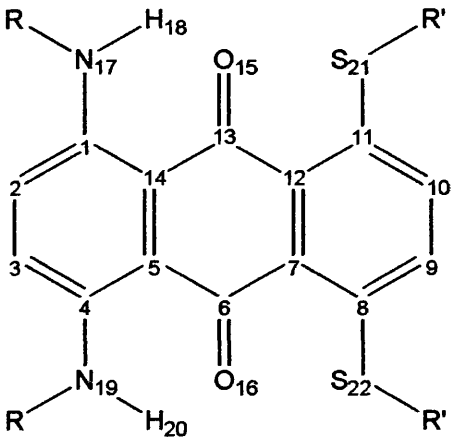
Table 5-20. Calculated properties of selected 1,4-bis(amino)-5,8-(sulfanyl)anthracene-9,10-diones using the AM1 method



R	$CH_2CH(CH_3)_2$	$CH_2CH(CH_3)_2$	$(CH_2)_2\ CH_3$	$(CH_2)_2\ CH_3$	$(CH_2)_2\ OH$	$(CH_2)_2\ OH$	C_6H_5	C_6H_5	H
R'	$CH_2\ CH_3$	C_6H_5	$CH_2\ CH_3$	C_6H_5	$CH_2\ CH_3$	C_6H_5	C_6H_5	$CH_2\ CH_3$	$CH_2\ CH_3$
	(140)	(139)	(133)	(134)	(142)	(141)	(143)	(144)	(148)
Distance [a]									
C1N17	1.410	1.379	1.376	1.382	1.376	1.383	1.384	1.399	1.393
H18N17	0.998	1.005	0.998	0.998	0.998	0.996	0.996	0.998	1.001
O15H18	1.976	1.945	1.971	1.966	1.969	1.961	1.949	1.959	1.962
O15C13	1.249	1.250	1.252	1.250	1.252	1.243	1.252	1.250	1.251
C11S22	1.685	1.687	1.779	1.687	1.681	1.727	1.705	1.677	1.684
Charge [b]									
O15	-0.311	-0.332	-0.327	-0.326	-0.332	-0.304	-0.333	-0.318	-0.322
O16	-0.328	-0.334	-0.323	-0.334	-0.325	-0.318	-0.334	-0.329	-0.322
N17	-0.280	-0.345	-0.342	-0.342	-0.353	-0.328	-0.295	-0.256	-0.270
N19	-0.334	-0.338	-0.342	-0.342	-0.357	-0.322	-0.296	-0.304	-0.270
S21	0.385	0.509	0.384	0.500	0.383	0.335	0.504	0.401	0.400
S22	0.382	0.501	0.405	0.509	0.349	0.335	0.498	0.370	0.400
H18	0.216	0.263	0.263	0.261	0.260	0.255	0.272	0.250	0.260
H20	0.262	0.261	0.263	0.262	0.261	0.250	0.273	0.276	0.260
HOMO [c]	-7.530	-7.339	-7.348	-7.365	-7.443	-7.740	-7.435	-7.493	-7.580
Dipole moment [d]	1.130	1.118	1.093	1.338	3.136	0.508	0.343	1.236	1.613
LUMO 1 [c]	-1.076	-1.062	-1.039	-1.084	-1.073	-1.271	-1.215	-1.178	-1.274
LUMO 2 [e]	0.924	0.938	0.961	0.916	0.927	0.729	0.785	0.822	0.726

[a] Bond lengths in angstroms. [b] Mopac charge [c] In eV. [d] Dipole moment in Debyes. [e] In eV, a factor of 2 eV has been added to make the LUMO energies positive.

Table 5-21. 1,4-bis(amino)-5,8-bis(sulfanyl)anthracene-9,10-diones using ab initio methods



Number	(148)	(133)	(134)
R	**H**	**$(CH_2)_2CH_3$**	**$(CH_2)_2CH_3$**
R'	**CH_2CH_3**	**CH_2CH_3**	**C_6H_5**
Basis set	**6-31G****	**6-31G****	**6-31G****
Distance [a]			
C1N17	1.361	1.363	1.362
C4N19	1.361	1.363	1.362
H18N17	0.991	0.991	0.991
H20N19	0.991	0.991	0.990
O15H18	1.860	1.880	1.901
O16H20	1.860	1.880	1.901
O15C13	1.210	1.207	1.207
O16C6	1.210	1.207	1.207
S21C11	1.770	1.788	1.799
S22C8	1.770	1.788	1.799
Charge [b]			
O15	-0.627	-0.629	-0.629
O16	-0.627	**-0.629**	-0.629
N17	-0.805	-0.828	-0.825
N19	-0.805	-0.828	-0.825
H18	0.362	0.363	0.360
H20	0.362	0.363	0.360
S24	0.314	0.314	0.408
S23	0.314	0.314	0.408
Total energy [c]	*-1745.8*	-1980.0	2282.0
Dipole moment [d]	0.2404	0.107	0.996
HOMO [e]	-6.858	-6.634	-6.729
LUMO [e]	1.040	1.046	0.838

[a] Bond lengths in angstroms. [b] Bond angles in degrees. [c] In A.U. [d] Dipole moment in Debyes [e] In eV

5.6.4 Hetero cyclic anthracene-9,10-diones

The charge on the nitrogen atom N24 in the thiazine ring is reduced, when compared to Mitoxantrone (**24b**) which carries a charge on N24 of -0.34 compared with -0.28 for 7-{[2-(dimethylamino)ethyl]amino}14H-naptho[2,3a]phenothiazine-8,13-dione (**151f**) (Table 5-22), however the other nitrogen atom N21 is unaffected with a value of -0.34 which is in line with Mitoxantrone (**24b**).

The ionisation potential for the thiazine derivatives is reduced in comparison to Mitoxantrone (**24b**), for example 7-{[2-(dimethylamino)ethyl]amino}14H-naptho[2,3a]phenothiazine-8,13-dione (**151f**) has a value of 7.43 eV, which compares to 7.71 eV for Mitoxantrone (**24b**).

The introduction of a thiazine ring into the system leads to no real effect on the energy of the adjusted LUMO with values of 0.68 eV for 7-{[2-(dimethylamino)ethyl]amino}14H-naptho[2,3a]phenothiazine-8,13-dione (**151f**), this compares to 0.71 eV for Mitoxantrone (**24b**) (Table 5-22).

The thiazine ring reduces the atomic charge on the nitrogen atom within the ring, to values similar for the exocyclic oxygen atom present in both Mitoxantrone (**24b**) and 1,4-dihydroxyanthracene-9,10-dione (**111**) this may not be desirable. However, the ionisation potentials are low, indicating an ease of the molecules to undergo oxidation and form polar species. The LUMO is unaffected in comparison to Mitoxantrone (**24b**).

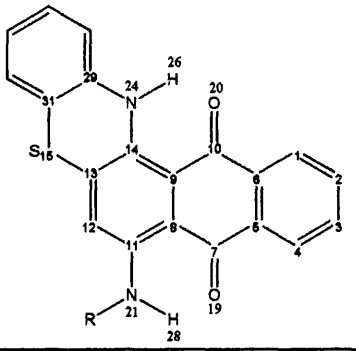
5.6.5 Conclusion

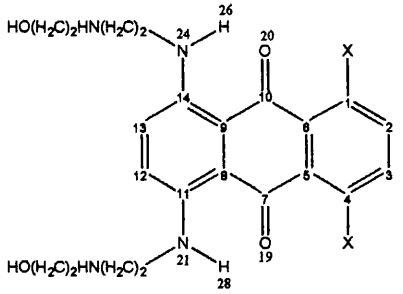
There are a number of differences between the ionisation potentials of the biologically active molecules such as Mitoxantrone (24b) and the inactive molecules such as anthracene-9,10-dione. The calculated values suggest that anticancer anthracene-9,10-dione molecule should have ionisation potentials in the region 7.78 to 7.95 eV. In addition, it seems that the

molecules should also possess basic atoms which can perhaps complex with DNA. Clearly the nitrogen atoms in Mitoxantrone (**24b**) and Amentantrone (**24a**) carry a much larger charge than the oxygen atoms in 1,4-dihydroxyanthracene-9,10-dione (**111**). It is suggested therefore that the active anthracene-9,10-dione molecules should not only possess basic centres but also have the appropriate ionisation potentials.

Many of the molecules synthesised in this work meet both these criteria and may possess anticancer activity, however this aspect of the work was beyond the scope of the current studies.

Table 5-22. Calculated properties of selected hetero anthracene-9,10-diones vs. selected 1,4-bis(aminoalkyl)anthracene-9,10-diones using the AM1 method





(24a) X=H
(24b) X=OH

R	$(CH_2)_2CH_3$	$(CH_2)_2OH$	$(CH_2)_2N(CH_3)_2$	Mitoxantrone	Amentantrone
	(151e)	(151g)	(151f)	(24b)	(24a)
Distance [a]					
N24-C14	1.393	1.394	1.394	1.375	1.397
N21-C11	1.374	1.374	1.374	1.373	1.391
O20-H26	1.936	1.934	1.934	1.960	2.098
O19-H28	1.969	1.958	1.961	1.943	2.042
C7-O19	1.249	1.248	1.248	1.252	1.240
C10-O20	1.245	1.247	1.247	1.250	1.242
S15-C13	1.695	1.690	1.690	N/A	N/A
S15-C31	1.689	1.686	1.687	N/A	N/A
Charge [b]					
O19	-0.345	-0.336	-0.336	-0.387	-0.310
O20	-0.335	-0.348	-0.345	-0.381	-0.315
N21	-0.331	-0.341	-0.336	-0.338	-0.294
N24	-0.283	-0.283	-0.284	-0.334	-0.301
H26	0.275	0.274	0.274	0.262	0.221
H28	0.266	0.264	0.266	0.262	0.234
S15	0.400	0.412	0.407		
HOMO [c]	7.595	7.430	7.429	-7.707	-7.952
Dipole moment [d]	2.439	1.339	1.075	5.548	1.631
LUMO 1 [c]	-1.285	-1.333	-1.321	-1.291	-1.038
LUMO 2 [e]	0.715	0.667	0.679	0.709	0.962

[a] Bond lengths in angstroms. [b] Mopac charge [c] In eV. [d] Dipole moment in Debyes [e] In eV, a factor of 2 eV has been added to make the LUMO energies positive.

5.7 References

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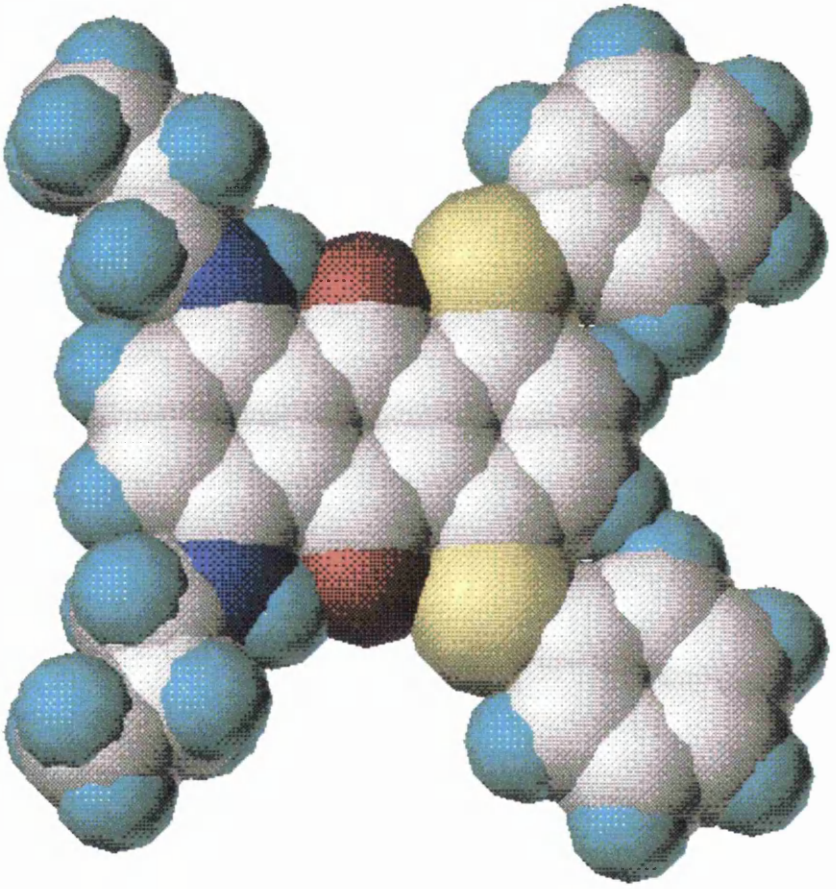
CHAPTER Six



Conclusions

6 Summary of the work and conclusion

The main aims of this research which are discussed in detail in chapter 2, were to synthesis some novel sulfur containing anthraquinone derivatives. Two classes of derivatives were successfully synthesised, i.e. 1,4-bis(amino)-5,8-(sulfanyl)anthracene-9,10-dione and 7-(alkylamino)-14H-naptho[2,3a]phenothiazine-8,13-dione.

Synthesis of 1,4-bis(amino)-5,8-(sulfanyl)anthracene-9,10-dione was achieved from 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione. 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione was synthesised from the chlorination of 1,4-dihydroxyanthracene-9,10-dione using a mixture of boric acid and 65% oleum with a catalyst of iodine. The thiolation of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione with thiophenolate, 2-methyl-2-propylthiolate and potassium thiocyanate under basic conditions did not result in the desired products i.e. the 1,4-dihydroxy-5,8-(sulfanyl)anthracene-9,10-diones. It was concluded that the lack of reaction was due to the deactivating effect of the hydroxyl groups, a similar effect was found in 1,4-dimethoxy-5,8-dichloroanthracene-9,10-dione.

Amination of 1,4-dihydroxy-5,8-dichloroanthracene-9,10-dione was achieved using the reduced form, 2,3-dihydro-9,10-dihydroxyanthracene-1,4-dione (**121**) with several alkyl amines. However, the reaction of 2,3-dihydro-9,10-dihydroxy-5,8-dichloroanthracene-1,4-dione (**121**) with aniline to yield 1,4-bis(phenylamino)-5,8-dichloroanthracene-9,10-dione (**131**) required the addition of boric acid to facilitate the reaction.
Once synthesized, the 1,4-bis(amino)-5,8-dichloroanthracene-9,10-diones were then thiolated with thiophenol and 2-ethanethiol in DMF with an appropriate base to yield the

1,4-bis(amino)-5,8-(sulfanyl)anthracene-9,10-dione. The thiolation of 1,4-bis(amino)-5,8-dichloroanthracene-9,10-diones either with potassium thiocyanate or with 2-methyl-2-propyl thiolate was unsuccessful. The thiolation of 1,4-bis{[2-(dimethylamino)ethyl]amino}-5,8-dichloroanthracene-9,10-dione (**127**) and 1,4-bis{[2-(diethylamino)ethyl]amino}-5,8-dichloroanthracene-9,10-dione (**128**) was also unsuccessful with thiophenolate and 2-ethanethiolate.

Prolonged reaction times for the thiolation of 1,4-bis(amino)-5,8-dichloroanthracene-9,10-diones in DMF led to dealkylation products. Dealkylation has been attributed to a free radical reaction involving oxygen, which attacks the hydrogen atom α to the amino group and results in the formation of peroxide that decomposes to yield the amine, water and a carbonyl compound. Dealkylation also occurred in the simpler 1,4-bis(alkylamino)anthracene-9,10-diones.

Synthesis of 7-(alkylamino)-14H-naptho[2,3a]phenothiazine-8,13-dione was obtained from the reaction of 1-(alkylamino)-4-hydroxyanthracene-9,10-diones with 2-aminothiophenol in the presence of boric acid. The direct amination of 7-hydroxy-14H-naptho[2,3a]phenothiazine-8,13-dione with alkylamines was unsuccessful, as was the alkylation of 7-amino-14H-naptho[2,3a]phenothiazine-8,13-dione with 2-chloroethanol.

Molecular modeling studies were carried out using the MNDO, AM1 and PM3 methods to assess the accuracy of these methods, they were used to calculate the geometries of 6 known anthracene-9,10-dione derivatives with known structures present in the Cambridge crystallographic database. The results showed that:

- Non-planar structures were predicted for 1,4-bis(alkylamino)- and 1,4-bis(phenylamino)-anthracene-9,10-diones which are contrary to the X-ray crystallographic data. This non planar geometry has been attributed in each method to the amino group preferring a lower energy sp^3 tetrahedral conformation rather than a planar sp^2 trigonal conformation. This results in the carbonyl oxygen atom being pushed out of plane of the molecule to maintain the hydrogen carbonyl oxygen bond distance.
- Planar geometries are produced for each molecule however, when the heavy atoms are constrained to lie in one plane, though this leads to a small energy penalty.
- The PM3 method is more accurate at predicting the hydrogen oxygen bond distance but the AM1 method is more accurate at predicting the heteroatom carbon atom bond lengths. The MNDO method is the least accurate
- The AM1 method is better than the PM3 and MNDO methods for modeling of 1-[6-(sulfanylhexyl)thio]anthracene-9,10-dione as it gives a planar geometry in line with the x-ray crystallographic data.

The AM1 method was selected to model the synthesized compounds as it gives the best overall.

As a comparison, some higher level ab initio calculations were also carried out on selected models. Although time consuming these calculations did maintain the planar geometry in line with crystallographic data.

The computational data highlighted two factors that may be significant in the biological activity of anthracene-9,10-diones. These factors are the HOMO or ionization potential of the molecules and the atomic charge of exocyclic atoms. It was concluded that molecules which are biologically active possess basic nitrogen atoms and ionization potentials calculated by the AM1 method in the range of 7.7-7.9 eV. The computational data of the derivatives synthesized in this work shows many of them meet this criteria

and therefore may possess anti-cancer activity, though this aspect of the work was beyond the scope of the current studies.

Overall a set of twenty four anthracene-9,10-dione derivatives were synthesized, of these 19 were novel and twelve of them contained sulfur. The computational results indicate that the molecules synthesized have properties similar to biologically active derivatives such as Mitoxantrone (**24b**) as opposed to the inactive derivatives such as anthracene-9,10-dione (**1**) and 1,4-dihydroxyanthracene-9,10-dione (**111**).

APPENDIX

The following table defines the keywords commonly used in the semi-empirical calculations performed for this research.

Keyword	*Definition*[1]
AM1	Use the Austin Model 1 Hamiltonian
DDMIN = *n.nn*	The minimum size of the trust radius in EF calculations is set to 0.001 if this keyword is not used. Various sizes of the trust radius can be defined by setting DDMIN = *n.nn*
EF	The eigenvector following routine is used in the search for an energy minimum.
GEO-OK	Overrides the interatomic distance check.
LET	Override certain safety function (used in conjunction with DDMIN).
NOINTER	Do not print interatomic distances.
NOLOG	Suppress the generation of a log file.
NOMM	Do not use molecular mechanics correction to C(O)NH bonds.
PM3	Use the MNDO-PM3 Hamiltonian.
PRECISE	Criteria to be increased by 100 times.
VECTORS	The eigenvectors are to be printed.
XYZ	Do all geometric operations in Cartesian coordinates.

[1] Adapted from the *MOPAC 93 manual*, Fujitsu Ltd., Tokyo, Japan, 1993.